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Warren Taylor Vaughan

1893-1944

ALL too soon, death came, as it does to all men, great and small, to Warren Taylor Vaughan on April 2, 1944, in the early morning. Death struck with lightning swiftness and halted the dictations of thoughts from one of the keenest contemporary minds and one of the most prolific pens of the past two decades. Warren Vaughan had earned for himself the rare title of "The famous son of a famous father." He was the youngest of the five sons of Victor C. Vaughan, dean of the University of Michigan Department of Medicine and Surgery for thirty years, founder and editor of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE. He followed in his father's footsteps and carried on in the editorship of this Journal in enviable style. He was one of the founders and an associate editor of *The Journal of Allergy*. He has contributed about two hundred articles to current medical literature.

Warren Vaughan's opus magnum, *Practice of Allergy* (1939), is outstanding in its field. His *Primer of Allergy* (1939, 1944) and a popular best seller, *Strange Malady*, both for the laymen, have been boons to sufferers. He was a pioneer internist allergist, worked untiringly for the application of the scientific principles of immunology, and wrote the early text on *Allergy and Applied Immunology* (1931). He has thus contributed immeasurably to the scientific development of this field. He was a founder, secretary general and treasurer (1928-1938), and in 1939 president of the American Association for the Study of Allergy. In the same year he held the presidency of the Society for the Study of Asthma and Allied Conditions. He was a member of the American Rheumatism Association, the Society of Investigative Dermatology, the International Society for Gastroenterology, and an honorary member of the Institute for the Practice of Medicine, Barcelona, Spain, and the Society for the Study of Allergy, Argentina. He served as a member of the councils of many nationally important organizations.

He received honors from various medical and honorary societies, and his Alma Mater bestowed upon him the honorary degree of Master of Science at

the Alumni Convocation on Founders' Day, October 4, 1941. At this time he delivered a very erudite and philosophic paper upon his unusual philosophy of life and his conceptions of duties and ideals. He was a crusader for the establishment and the maintenance of high ideals and high standards of practice and of research especially in his highly specialized field of medical practice. He was a leader, not only in allergy, but also in the broad field of internal medicine in which he was certified as a specialist by the American Board of Internal Medicine. In this Journal he wielded an influence for improved close relationships between laboratory and clinical medicine. He inspired his contributors in both experimental and clinical circles.

Warren Vaughan had an unusual ability for leadership that was evident even in his college days. In the medical school classes he was a great stimulus to all who had the privilege of knowing him. I was one of those fortunate enough to be close to him. His habits were exemplary. He encouraged the highest scholarship and accomplished the same for himself. In definitely unsolvable controversial matters, he took a sane middle-of-the-road attitude. He was an ardent supporter of whatever he deemed was right and was sharply critical of wrong, unethical, and dishonest actions. In his medical school days he was elected to the honorary fraternities of Alpha Omega Alpha and Sigma Xi. In his college days he was active in improving the standards of the fraternities. He was a member of Beta Theta Pi and Phi Rho Sigma of which he was author of the ritual and a councilor for many years.

Upon graduation from the University of Michigan in 1916 he served as house officer at the Peter Bent Brigham Hospital and won such distinction that ever afterward Michigan men had a definite advantage when they applied for service under "The Professor." Dr. Henry A. Christian was always his ideal of the master clinician. On completion of his house officership he accepted a position on the Harvard Medical School faculty in the Department of Public Health and Hygiene under Milton J. Rosenau. He took advantage of the abundant opportunities for the practice of preventive medicine in the field of internal medicine. When World War I was declared, he entered the Armed Forces and rose rapidly through all grades from the rank of first lieutenant to that of lieutenant colonel and chief of the medical service Camp Hospital 41 American Expeditionary Forces, Is-sur-tille, France. He returned to civilian life and practice in 1920 and chose Richmond, Virginia, as the place in which to do his lifework. He was director of the Vaughan-Graham Clinic. He contributed greatly to the high position of the practice of the art and especially of the science of medicine in that community.

Warren Vaughan assumed various editorial duties; in addition to his editorship of this Journal and associate editorship of *The Journal of Allergy*, he acted as collaborating editor of *Folia clinica chimica et microscopica* of Bologna, Italy. He was on the editorial board of *The American Journal of Digestive Diseases* and the *American Journal of Clinical Pathology*. He was a former member of the editorial board of *The American Journal of Syphilis, Gonorrhea, and Venereal Diseases*, and of *The Review of Gastroenterology*.

He was a member of the Committee on the Cost of Medical Care; a member of the Committees of Aerobiology and of Food Habits of the National Research Council; member since 1918 and, since 1938 on the council, of the American

Association for the Advancement of Science; member of the Research Council on Problems of Alcohol. He was active in the American Society of Clinical Pathologists, the Medical Society of Virginia (vice-president 1931-1932), Virginia Academy of Sciences, the Southern Medical Association, and the American Medical Association. He was a member of the Commonwealth Club.

In 1917 he married Emma Elizabeth Heath. To them were born four sons, Victor Clarence III, Warren Taylor, Jr., John Heath, and David DuPuy. His sons are carrying on the family tradition, the two older having received their medical degrees from Harvard Medical School and the two younger being students there.

Warren Vaughan will be greatly missed by every one of his many friends who have depended upon him for wise council. He died entirely too soon. We are prepared to accept as inevitable the passing of our older teachers, but we irreligiously protest the premature passing of our contemporaries just entering upon the promising harvest years of life. The abrupt premature closing of Warren Vaughan's scientific and medical career and his editorial work is bitterly lamented, but his personal charm, his inspiring interest in men and in science will long be remembered and will stimulate many to carry on in the high tradition that he forcefully exemplified.

—*George Herrmann.*

CLINICAL AND EXPERIMENTAL

THE EFFECT OF CHEMOTHERAPEUTIC SULFA DRUGS UPON THE GROWTH OF α-HEMOLYTICUS STREPTOCOCCUS*

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THE recovery of a few patients from subacute bacterial endocarditis when treated by combined fever and chemical therapies[1] suggested some investigations as to the rationale of this method of treatment. With this object in mind, attempts were made to determine whether the susceptibility of α-hemolyticus Streptococcus, commonly found in this disease, could be modified in vitro by varying the conditions of cultivation, with special reference to the temperature of incubation. In order to study closely this relationship, it was deemed advisable to find the requirements for growth of the strain selected. Furthermore, a method[2] was developed to bring the drugs tested into continuous contact with the microorganisms by bubbling a suitable mixture of gases through the cultures during incubation. This method served also to assure a uniform temperature of the medium. By the use of various media it was possible to obtain growth curves, differing in the duration of the lag period, the phase of logarithmic growth, and the phase of decline. The results of the studies on the effect of sulfonamides on growth of α-hemolyticus Streptococcus under various conditions are embodied in this paper.

EXPERIMENTAL

A *strain* of α-hemolyticus Streptococcus, obtained from the blood of a patient suffering from subacute bacterial endocarditis, is the microorganism used throughout these experiments. The strain failed to ferment esculin, was killed by exposure at 60° C. for half an hour, and was bile-insoluble. It was maintained by daily transplants in 1 per cent glucose broth incubated at 37.5° C. The cultures showed spontaneous agglutination forming granular deposits at the bottom and along the sides of the tube. After twenty-four hours of incubation, colonies appearing on the surface of "pour" plates of 1 per cent glucose agar and rabbit blood were discrete, small, slightly raised, and granular. In forty-eight hours the colonies became larger and more irregular. Biweekly serial transplants of supernatant of glucose-broth cultures into "buffer" broth were made, care being exercised to avoid transfer of the granular deposits. The "buffer" veal infusion broth contained 1 per cent peptone (Fairchild) and 0.2 per cent sodium phosphate at final pH 7.4. Thus, after two transplants, the

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colonies on agar plates appeared finely granular and the broth cultures uniformly turbid. The "smooth" characteristics of the cultures lasted for approximately four days; later a reversal to previous appearance was observed in all cultures.

1. STUDIES ON OPTIMUM REQUIREMENTS FOR CULTIVATION OF THE STRAIN OF α-HEMOLYTICUS STREPTOCOCCUS USED

The purpose of these experiments was to determine the effect of various factors upon the growth of the strain selected before proceeding with the main studies. The following is a short description of the findings:

A. Chart I illustrates the effect of different gas mixtures upon the growth of the strain of α-hemolyticus Streptococcus in 1 per cent glucose broth at 37.5° C. and initial pH 7.6.

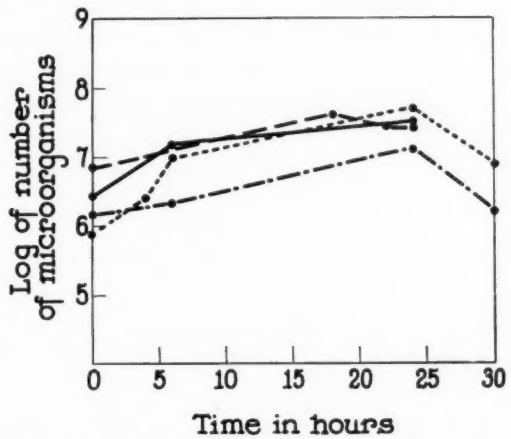

Chart I.—The effect of gas mixtures upon the growth of *Streptococcus viridans*.

.—.—.—.—.—.— 10% CO_2
---------- 10% CO_2, 5% O_2, 85% N_2
———— 5% CO_2, 95% O_2
— — — — — — — — 25% CO_2, 75% O_2

Incubation temperature 37.5 C. Medium 1 per cent glucose broth pH 7.6.

Chart I illustrates the growth curves obtained with various gas mixtures. The mixture consisting of 10 per cent CO_2, 5 per cent O_2, and 85 per cent N_2 produced a short lag period. The active logarithmic phase began in less than five hours following inoculation and continued for a period of twenty hours. This gas mixture was employed in all the experiments about to be reported in this paper. The apparatus previously described was used.[2] A series of culture tubes was always immersed at a depth where the level of the fluid in the tubes was below the level of the water in the water bath. Each tube containing 30 c.c. of 0.4 per cent glucose broth and seeded with 1 c.c. of an 18-hour broth culture of the microorganism was maintained in the water bath at the desired temperature. The mixture of gases was constantly bubbled through the media at a regulated speed, keeping the microorganisms in constant agitation and in immediate contact with the medium. The pH range used was 7.4 to 7.8. The bacterial counts were made by determining the number of colonies on "pour" plates of 1 per cent glucose agar and rabbit blood, seeded with 1 c.c. of the material tested at 6-, 24-, and 30-hour intervals.

B. In experiments described below, α-hemolyticus Streptococcus was grown in various media incubated at different temperatures as indicated in Charts II and III.

One cubic centimeter of an 18-hour broth culture of the microorganism was seeded into each medium, and the gas mixture was bubbled through the media. Here again, samples were removed for bacterial counts at the beginning of the experiment and at 6-, 24-, and 30-hour intervals.

The results recorded in Charts II and III bring out the fact that the strain of α-hemolyticus Streptococcus used may be cultivated at a wide temperature range. The higher the temperature, the shorter is the lag period. On the other hand, at 37.5° C. the logarithmic phase is of longer duration and the decline is slower than in the same media at higher temperature. The phase of decline roughly but consistently parallels the drop in the pH of the cultures. For this reason, the longest logarithmic phase is obtained in the best buffered medium containing rabbit serum and the most rapid decline in media containing glucose. Having completed the observations on the cultivation of α-hemolyticus Streptococcus under various conditions, experiments were carried out on the effect of certain sulfa drugs upon the growth of this strain.

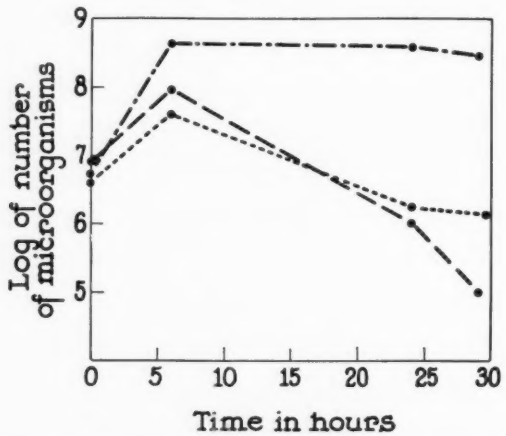

Chart II.—The effect of temperature upon the growth of *Streptococcus viridans*.

---------- 39 C
— — — — — — — — — 39.5 C
.—.—.—.—.—.— 40 C

Gas mixture: 10 per cent CO_2 + 5 per cent O_2 + 8 per cent N_2. Medium: 0.4 per cent glucose broth pH 7.4.

2. THE EFFECT OF SULFA DRUGS UPON THE GROWTH OF α-HEMOLYTICUS STREPTOCOCCUS

A. Various amounts of *sulfanilamide* were added to 30 c.c. of 0.4 per cent glucose broth of initial pH 7.4, thus giving final concentrations of 1:1500, 1:2000, 1:3000, and 1:6000. The tubes containing the mixtures were then seeded each with 1 c.c. of an 18-hour broth culture of the microorganism. The gas was bubbled through at a regulated speed. The temperatures tested were 39°, 39.5°, 40°, 40.5°, 41°, and 41.5° C. At the beginning of the experiment and after intervals of 6, 24, and 30 hours, samples were removed for bacterial counts.

It is seen from Chart IV that the various concentrations of the drug tested at various temperatures had no significant effect upon the growth of α-hemolyticus Streptococcus. The growth curves were quite similar to the respective controls.

B. The effect of *various sulfa compounds including sulfanilamide* on the α-hemolyticus Streptococcus was tested as follows:

Series Number 1

Tube 1, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum + 10 mg. sulfanilamide

Tube 2, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum + 10 mg. sodium sulfapyridine

Tube 3, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum + 10 mg. sodium sulfathiazole

Tube 4, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum (control)

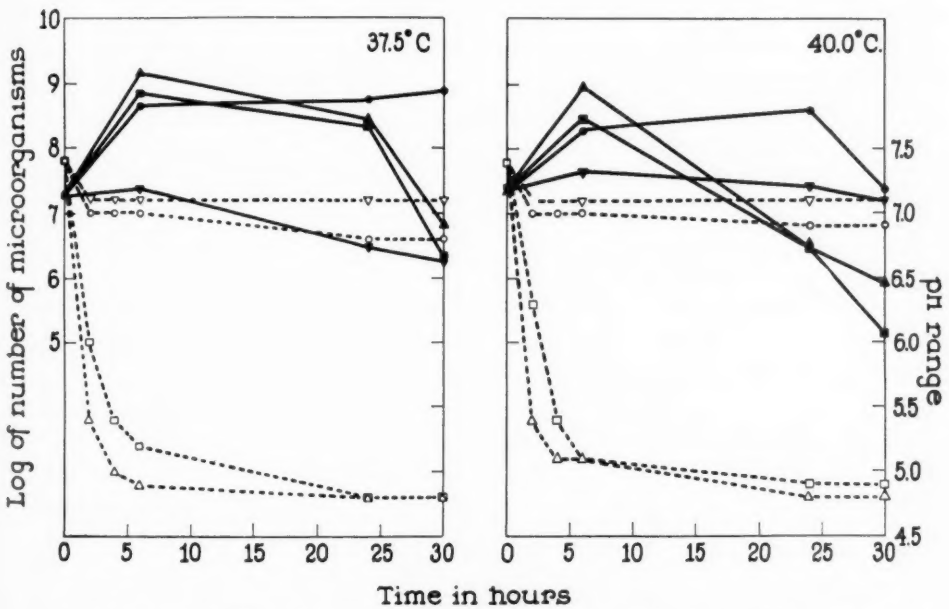

Chart III.—The effect of temperature upon the growth of *Streptococcus viridans* in various media.

Plain broth—Rabbit Serum
1 per cent Glucose Broth
1 per cent Glucose Broth—Rabbit Serum
Plain Broth

Gas mixture: 10 per cent CO_2 + 5 per cent O_2 +85 per cent N_2.

Series Number 2

Tube 1, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum + 10 mg. sulfadiazine

Tube 2, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum + 10 mg. sulfaguanidine

Tube 3, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum + 10 mg. promin

Tube 4, 30 c.c. plain broth + 1.5 c.c. inactivated rabbit serum (control)

The final concentration of sulfa drugs in the media was 1:3150.

Each tube was seeded with 1 c.c. of an 18-hour broth culture of the microorganism. The initial pH was 7.4, the gas mixture was constantly bubbled through, and the temperatures tested were 37.5° and 40° C. Samples were removed for bacterial counts at the beginning of the experiment and after 6-, 24-, and 30-hour intervals.

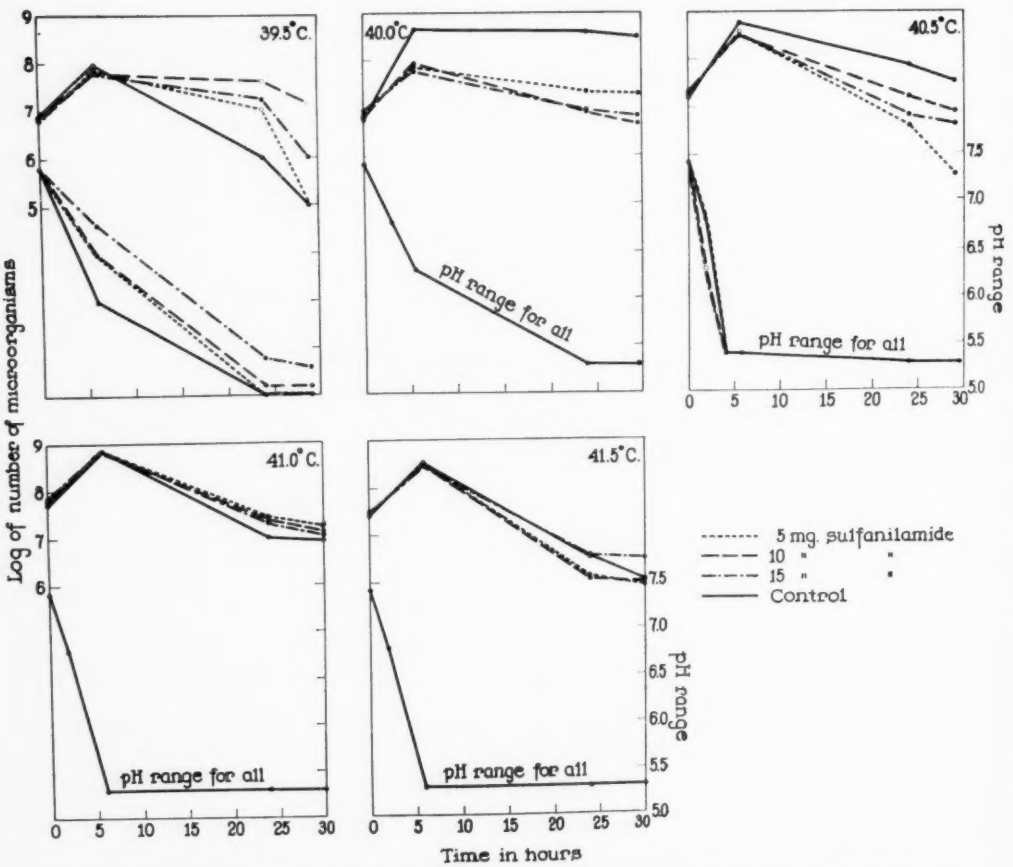

Chart IV.—The effect of various concentrations of sulfanilamide upon growth of *Streptococcus viridans* at various temperatures.

Gas mixture: 10 per cent CO_2 + 5 per cent O_2 + N_2. Medium: 0.4 per cent glucose broth pH 7.4.

Here again the sulfonamide compounds did not produce any appreciable effect upon the growth of the strain of α-hemolyticus Streptococcus tested. It may be seen from the examination of the counts of control cultures that various growth curves were obtained by the use of a wide temperature range and different media. The compounds tested failed, however, to inhibit the growth both under the least and the most suitable conditions of cultivation.

COMMENT

In the work embodied in this paper, the effect of various sulfa compounds upon the growth of a strain of α-hemolyticus Streptococcus isolated from the blood of a patient suffering from subacute bacterial endocarditis was determined by counting viable microorganisms in samples removed at frequent intervals from control and experimental cultures. The experimental setup used here was the same as previously described.[2] It permitted the frequent removal of samples without any interference with the growth and without change in the incubation temperature. It was accomplished by raising slightly the pressure of the gas mixture continuously bubbled through the culture and siphoning off the medium through a side tube. The gas mixtures used were selected after ascertaining their favorable effect upon growth. They were also responsible for continuous agitation of the cultures. Thus, the drugs tested were brought into constant contact with the microorganism. The agitation was considered important in the work in which α-hemolyticus Streptococcus, having a strong tendency to form clumps and settle at the bottom of the fluid medium cultures, had to be employed. Incubation of cultures in a water bath with a sensitive thermoregulating mechanism and constant stirring* offered an opportunity for reliable and accurate temperature control which could be easily and quickly changed to any desired level. The different features of the method of cultivation described were used for various studies by previous authors singly and in combinations; to mention only the most recent studies of Longsworth and MacInnes[3, 4] on stabilization of pH in bacterial cultures; of Carpenter and Wingate[5] on cultivation of microorganisms at various temperatures; and of Magoon and Brunstetter[6] on cultivation of bacteria in constantly agitated media. It may be stated, however, that the combination of the various features in a somewhat simplified form served well the particular investigation described in this paper.

With special reference to the problem of this investigation, certain points should be emphasized.

The effects of sulfa compounds on α-hemolyticus Streptococcus (*Streptococcus viridans*) received considerable attention of a number of previous investigators:

It seems that there exist considerable variations in the susceptibility of different strains to this group of drugs. According to Bliss, Long, and Feinstone,[7] most of the α-hemolyticus Streptococcus strains have proved susceptible to 1:10,000 concentration of sulfanilamide. Some of the strains which were resistant were strains of *Streptococcus faecalis,* enterococcus being clearly resistant to the drugs (Neter[8]). According to Maegraith and Vollum,[9] the growth of α-hemolyticus Streptococcus was affected by the drugs only in the presence of leucocytes. Bratton[10] investigated bacteriostatic effects of the drugs upon three freshly isolated strains of α-hemolyticus Streptococcus and found no effect on two strains, while for the third strain sulfanilamide was bacteriostatic in a concentration of 1:1000 and bactericidal in a concentration of 1:500. Proseptasine had no effect; soluseptasine was bacteriostatic in 1.25 per cent and bac-

*Fisher Scientific Company, Constant Temperature Water Bath, Fisher Unitized Water Bath, Catalogue No. 15-444.

tericidal in 25 per cent; T 607 was bacteriostatic in a concentration of 1:4400; while T 626 was bacteriostatic in a concentration of 1:20,000 and bactericidal in a concentration of 1:10,000. Swain[11] determined the effect of 4:4′-diaminodiphenyl sulfone, sulfapyridine, and sulfanilamide upon four strains of α-hemolyticus Streptococcus isolated from cases of subacute bacterial endocarditis treated with the drugs. The two strains isolated from cases showing clinical improvement were susceptible, while the strains isolated from cases which failed to respond to the treatment were resistant to the drugs in vitro. Treadway[12] also reported that four strains of the α-hemolyticus Streptococcus showed wide variation in response to the drugs, sulfathiazole being the most effective.

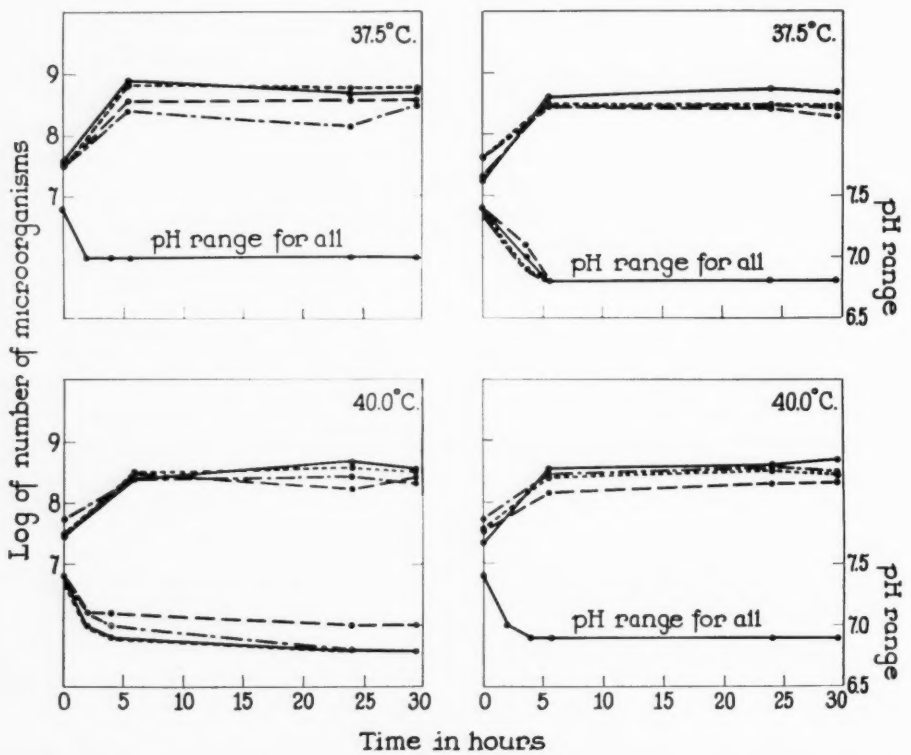

Chart V.—The effect of sulfanilamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfapromin, and sulfaguanidine upon growth of α-hemolyticus Streptococcus at various temperatures.

———	Control	———	Control
- - - - - -	Sulfadiazine	- - - - - -	Sulfanilamide
— — — — —	Sulfapromin	— — — — —	Sulfathiazole
.—.—.—.—	Sulfaguanidine	.—.—.—.—	Sulfapyridine

Gas Mixture: 10 per cent CO_2 + 5 per cent O_2 + 85 per cent N_2.

The cause of the variation in susceptibility of α-hemolyticus Streptococcus to the drugs in vitro still remains obscure. Gay, Clark, Street, and Miles[13] have reported that they were able to obtain killing of Streptococcus by sulfanilamide by 1:5000 concentrations in broth, when the cultures were incubated between 39 and 40° C. Bacteria grew more slowly and to a lower peak at 40° C. than at 37° C.

In the investigation reported in this paper a single strain, freshly isolated from a human case of subacute bacterial endocarditis, was studied. The strain proved at once resistant to the effect of drugs under the ordinary conditions of cultivation. A series of experiments was then carried out to determine whether this resistance could be changed under modified conditions of cultivation, i.e., various hydrogen-ion concentrations during the logarithmic and stationary phase of growth; the use of more and less favorable media; and finally the employment of various temperatures compatible with growth. In this manner, several types of growth curves were obtained; these differed in the duration and the level of the lag period, of the phase of logarithmic growth, and of the stationary stage. Under the conditions tested, there was only insignificant bacteriostatic, and no clear-cut bactericidal effect obtained by the use of sulfanilamide, sodium sulfathiazole, sodium sulfapyridine, sulfaguanidine, sulfadiazine, and promin.

SUMMARY

The inherent resistance to sulfanilamide and various sulfa compounds of a strain of α-Streptococcus hemolyticus isolated from a human case of subacute bacterial endocarditis remained unchanged under both favorable and adverse conditions of cultivation in vitro.

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EFFECT OF CERTAIN SALTS ON ACTIVITY OF SULFACETAMIDE*

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IT IS common knowledge that sodium citrate solutions are widely used in bladder irrigations after transurethral surgery because of their anticoagulant property. For obvious reasons, one of us (B. D. P.) wished to add sulfacetamide to this irrigating solution. Before clinical use, however, it seemed wise to investigate the bacteriologic behavior of the mixture inasmuch as sulfonamide derivatives are known to encounter "blocking" of their bacteriostatic activity in the presence of various agents, such as para-aminobenzoic acid, peptones, methionine, and sugar.

The first experiment with citrate and sulfacetamide produced such unexpected results that it was decided to employ two other salts for comparative purposes. Sodium acetate and sodium lactate were selected as representing anions which the drug must frequently meet in the body. However, stronger solutions than those present under physiologic conditions were used for study. Since the 4 per cent sodium citrate solution is the one employed in urologic irrigations, this strength was prepared and the other two salts were made of equimolar concentration.

METHOD

Aqueous or dextrose broth solutions of each salt were prepared. The test sulfonamide was added to these in amounts equal to saturation in water. Aliquots (2.5 c.c.) were inoculated with 0.025 c.c. (0.1 c.c. of 1:4 dilution) of 18-hour dextrose broth cultures of the organisms. Control tubes were of two types: those containing solutions of the salts only, and those containing the sulfonamide solution alone. After 4- and 24-hour incubation periods, cultures were made for bacterial counts by plating in meat infusion agar to which para-aminobenzoic acid had been added. One-tenth c.c. and 0.1 c.c. of 1:100 dilution were used for plating. All solutions were tested for sterility.

RESULTS

Sulfacetamide is regarded as an adequate bactericidal opponent of *Escherichia coli* and is frequently employed in urinary tract infections. In these experiments, recently isolated strains of *E. coli* were employed. All strains used gave typical reactions to the carbohydrates and were methyl red and indole positive. In the control tests using aqueous solutions of acetate, lactate, and citrate, the organisms grew without restraint, and innumerable colonies of *E. coli* were produced on all plates, both in the 4- and 24-hour cultures. Sulfacetamide in aqueous solution was found to give complete sterilization in cultures of three of the four strains tested after 24 hours' incubation. The fourth culture showed four colonies of *E. coli* in 0.1 c.c. after 24 hours. In tubes containing sulfacetamide with acetate, innumerable colonies of *E. coli* were produced in both the 4- and 24-hour platings. When the cultures were made omit-

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ting the para-aminobenzoic acid, inhibition of growth of the *E. coli* was demonstrated, denoting a bacteriostatic rather than a bactericidal action. The bactericidal potency of the drug did not seem to be impaired appreciably by the presence of sodium lactate, all cultures showing a striking diminution of growth in 24 hours. This was true when cultured both with and without the addition of para-aminobenzoic acid to the media. The same results could not be repeated when sodium citrate was added with sulfacetamide to the culture media; in this instance bacterial growth was unimpaired and innumerable colonies of *E. coli* were produced in 0.1 c.c. of 1:100 dilution in both the 4- and 24-hour cultures. The omission of para-aminobenzoic acid did not alter this finding. The interference of citrate with the antibacterial action of sulfacetamide was thus clearly demonstrated, and the drug was shown to be rendered inert in the presence of citrate.

For purposes of comparison, it was deemed advisable to establish the effect of these salts on other organisms. Therefore, freshly isolated strains of *Staphylococcus aureus* and *Streptococcus hemolyticus* were employed with the particular sulfonamide considered efficacious against that organism. Sulfathiazole was tested with staphylococci. The same method of preparing tubes for incubation was used with the single exception that meat infusion broth was employed and the appropriate sulfonamide dissolved in it.

Four recently isolated strains of *Staphylococcus aureus* were employed, and cultures were prepared in the method indicated. In the experiments where the test salts were acetate and lactate, the organisms survived unaltered. Even the addition of sulfathiazole failed to restrict perceptibly the colony count. When sulfathiazole alone was used, all the cultures disclosed innumerable colonies at 4 and 24 hours. A marked inhibition in bacterial growth was demonstrated, however, in the tests containing sodium citrate and the mixture of citrate and sulfathiazole. There was not a noteworthy difference between the control containing citrate alone and the mixture with the sulfonamide. Although the latter colony count was somewhat lower, it was not significantly so.

A similar result was obtained, using in the same manner three freshly isolated strains of hemolytic streptococci against sulfanilamide. Consistent observations were made, although the susceptibility of the various strains of streptococcus to the sulfanilamide was irregular. The results noted with the staphylococci were duplicated in these tests. The streptococci were inhibited by the solution of citrate in broth, and the inhibitory action was strikingly apparent in all studies in which mixtures of citrate and sulfanilamide were used. In one case complete sterilization was obtained. These preliminary studies indicate that sodium citrate, when added to culture media, inhibits the growth of staphylococcus and streptococcus organisms. Sodium acetate and sodium lactate display no conspicuous inhibition or acceleration of sulfonamide activity. The drug behaves with complete indifference to their presence.

SUMMARY

The presence of sodium citrate (4 per cent strength) in a medium containing sulfacetamide nullifies the antibacterial behavior of the sulfonamide and permits unarrested development of *E. coli*. The citrate alone does not inhibit the growth of these organisms. The bacteriostatic potency of the drug is not similarly impaired by sodium acetate or sodium lactate.

AN INTRADERMAL TEST FOR VITAMIN C SUBNUTRITION*

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PRELIMINARY REPORT

THE test is based on the intradermal injection of a dye which will be decolorized by vitamin C. Slow decolorization of the dye would indicate that the tissues have an insufficient amount of vitamin C. Rapid decolorization would indicate a sufficient amount. A skin test is cheap, easy to perform, and quickly read. If it should reliably point out vitamin C subnutrition states, it would be a valuable addition to our armamentarium.

Up to now, the reported investigations have been on a method and technique suggested by Rotter[1] in 1937. He used 0.01 c.c. of a N/400 solution of dichlorphenol indophenol to raise a blue wheal of about 2 mm. in diameter. Rotter believed that if a wheal disappeared in less than 5 minutes, the tissues were saturated with vitamin C; that if the reduction time was from 5 to 10 minutes there was a normal amount of vitamin C in the tissues; and that there was a vitamin C deficiency if the reduction time was more than 10 minutes. A sharp division of opinion has been found in the subsequent reports of other investigators as to the efficacy of this particular test.

The following investigators have reported that this test was reliable. Portnoy and Wilkinson[2] found the method to be of value and thought there was a general correlation with blood vitamin C levels. Banerjee and Guha[3] found the intradermal test to be accurate for the assessment of the level of vitamin C nutrition in guinea pigs tested under controlled conditions. They also found good correlation between this skin test and the urinary excretion of vitamin C in nine healthy human subjects. Suzuki[4] reported that this intradermal test paralleled closely the blood vitamin C levels. Beck and Krieger[5] stated that this intradermal test and the saturation test for vitamin C corresponded closely. Slavich and Torrini[6] showed that the time required for decolorization in an individual was markedly decreased following intravenous injections of two to five hundred milligrams of vitamin C. Masuzawa[7] declared that this skin test was satisfactory in determining vitamin C deficiency. Reddy and Sastry[8] noted that the skin test corresponded well with dietary intakes and urinary excretion.

The following investigators have reported this intradermal test as described by Rotter to be entirely unreliable. Jetter,[9] Poncher and Stubenrauch,[10] Bakhsh et al.,[11] and Goldsmith et al.[12] reported independently that there was no correlation between the fasting blood ascorbic acid and this intradermal test. Gambigliani Zoccoli and Lombardo[13, 14] found this test unsatisfactory for diagnosis of vitamin C deficiency. Baraldi[15] and Cera[16] too concluded independently that this method was not sufficiently specific for its proposed use.

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Lima[17] found no correlation between this intradermal test and vitamin C saturation tests. Wright and MacLenathen[18] did not consider this intradermal test a reliable guide to the body vitamin C nutrition. They found the variations in the decoloration in the same patient too great to set up a normal range and that readings at different sites frequently varied considerably. Poulsen and Lieck[19] too found different results in the same patient in different arms and legs and did not feel the test was of practical value in the form advocated by Rotter.

The contradictory results reported by the various investigators of the Rotter test suggested that the test was not entirely satisfactory in that form. We planned to study the intradermal test in all its aspects. The study was not to be limited to the method described by Rotter. Different methods and techniques were to be investigated in an attempt to find one that would be a reliable guide to the body vitamin C nutrition.

ANIMAL EXPERIMENTS

The rabbit is able to synthesize its own vitamin C and keeps a fairly constant degree of vitamin C tissue saturation. The rabbit's abdomen, which has been shaved and cleaned, can be used for a large series of skin tests.

When as Rotter[1] suggested, 0.01 c.c. of a dichlorphenol indophenol solution was used to raise a wheal of about 2 mm., there were variations in the decolorization times among adjacent wheals. It was noted that the immediate area of needle puncture was a darker blue and took very much longer to decolorize than the rest of the wheal. This may be due to the destruction of vitamin C where the needle punctures the tissue, thus producing a lack of vitamin C at that point. With small wheals, this may have caused the variations. When larger wheals of about 4 mm. were raised, there was no longer any marked variation in the decolorization times, and the tests were easier to read. For example, five skin tests performed on Rabbit C at adjacent sites disappeared in times with a deviation from the average of less than 45 seconds. No attempt was made to regulate the amount of dye injected, but only the size of the wheal. In all subsequent animal work, wheals of about 4 mm. were used.

Different dilutions of dichlorphenol indophenol were used for the intradermal tests. The higher the dilution the shorter was the time required for decolorization. The more concentrated solutions required proportionately longer times for decolorization. (See Table I.) For example, in Rabbit M, an N/5000 solution was decolorized in 1.8 minutes while the N/500 took 12.8 minutes.

The intravenous administration of vitamin C reduced the time needed for decolorization in all rabbits. For example, in Rabbit R the intradermal test showed that a N/500 solution was decolorized in 9.8 minutes; after a 200 mg. intravenous injection the average time was 4.1 minutes. This shortened time was still present on the following day. It took six weeks for the decolorization time gradually to return to the preinjection time of 9.8 minutes.

RESULTS WITH HUMANS

When wheals of approximately 4 mm. were raised in the forearm, there was little variation in decolorization times in an individual. Five intradermal tests done at adjacent sites on the forearm in an individual disappeared in ap-

proximately the same time. The deviation from the average time was never more than 45 seconds. The 4 mm. wheal was used in all subsequent work.

Different dilutions of dichlorphenol indophenol were used for the intradermal tests. In an individual, the higher the dilution the shorter was the time proportionately required for decolorization. For example, in D.F., a N/300 solution was decolorized in 15.2, a N/600 in 8.4, a N/1000 in 6.3, and a N/1500 in 4.0 minutes. The problem was then to find a dilution with no toxic effect which would best differentiate in decolorization time between the various degrees of vitamin C tissue saturation. Preliminary skin tests in duplicate and triplicate with dilutions varying from N/1500 to N/350 were done on 167 patients. These were hospitalized children and adults. We knew approximately how much vitamin C was being ingested, and their vitamin C blood level was done at the same time as the skin tests.

TABLE I

RELATIONSHIP OF SKIN TEST TIME TO THE DILUTION OF THE DYE

RABBIT	DILUTION OF DYE	SKIN TEST TIME—MINUTES		
		I	*II*	*Average*
L	N/500	9.6	9.8	9.7
	N/1000	5.0	4.0	4.5
	N/2500	2.8	2.9	2.9
	N/5000	1.2	1.6	1.4
M	N/500	12.9	12.9	12.9
	N/1000	6.9	5.9	6.4
	N/2500	3.5	3.4	3.5
	N/5000	1.9	1.8	1.9
O	N/500	8.8	9.8	9.3
	N/1000	4.0	4.5	4.3
	N/2500	2.8	2.8	2.8
	N/5000	1.5	1.7	1.6

It became clear that the most concentrated solution that could be used without toxic effect would best differentiate between tissue saturation and unsaturation of vitamin C. There are at least two reasons for this. First, the concentrated solution takes a proportionately long time to become decolorized so that interpretation becomes easier. Thus, a difference between 10 and 15 minutes of using a concentrated solution would compare with 1 and 1½ minutes of using a very dilute solution. Second, there are other reducing substances in the skin such as glutathione whose effect tends to be minimized, the more concentrated the solution of dye that is used. The results with the N/300 solution of dichlorphenol indophenol, which is the most concentrated solution that we have used extensively, will be reported in detail.

A N/300 solution is prepared by dissolving 24 mg. of sodium 2-6 dichlorphenol indophenol with about 35 c.c. of boiling distilled water in a 50 c.c. volumetric flask, cooling, and making up to volume. This solution is prepared freshly every other day.

The technique is essentially the same as that used in the Mantoux and Schick tests. An ordinary tuberculin syringe and a short No. 26 needle are used. The syringe is rinsed twice with the dye before the injection. A part of the forearm without hair or veins is cleansed with alcohol and then permitted to dry. About 0.05 c.c. is injected into the skin in order to raise a wheal of approximately 4 mm. (3.5 to 4.5 mm.). The exact time of injection is noted from a

stop watch. Any excess dye or drop of blood which may obscure the reading is wiped off. The time when the blue color has disappeared altogether is noted. Where the needle enters the skin there may be a pin-point darker blue spot. This is not to be considered in determining the decolorization time. (See photograph.)

This method and technique was used on children and adults in the hospital. These were patients picked at random for the test. Two hundred and eighty-five tests were done in duplicate on the same and opposite forearms, and a blood vitamin C determination was performed on the patient at the same time. The approximate amount of vitamin C in their diet during the days preceding the test was known.

Fig. 1.—Technique of the intradermal test.

One hundred and two patients had a skin test time of from 3½ to 9 minutes, 104 from 9 to 13 minutes, 79 from 14 minutes to 25.6 minutes. The blood levels of vitamin C ranged from 0.0 mg. to 1.97 mg.

There were 59 blood levels under 0.3 mg.; the mean was 0.20 ± 0.07 mg. (See Table II.) The skin test time in 54 of these was 14 minutes or more; the other five were between 9.5 and 13.2 minutes; the mean was 16.7 ± 2.9 minutes.

TABLE II

COMPARISON OF BLOOD VITAMIN C LEVELS BELOW 0.3 MG. WITH SKIN TEST TIMES

CASE NO.	AGE YEARS	SEX	COLOR OF SKIN	BLOOD MG.	SKIN TEST TIME MINUTES		
					I	*II*	*Average*
1	7	M	W*	.00	17.2	16.4	16.8
2	7	M	W	.00	14.1	14.6	14.4
3	11	F	L.C.*	.10	20.2	20.4	20.3
4	9	F	W	.10	20.1	20.2	20.2
5	7	F	W	.10	18.5	18.6	18.6
6	8	F	C*	.11	25.5	25.7	25.6
7	8	F	L.C.	.11	19.9	19.2	19.6
8	2	M	C	.11	17.0	16.9	17.0
9	6	M	C	.11	16.5	16.5	16.5
10	7	M	W	.11	11.8	11.9	11.9
11	7	F	C	.12	19.8	----	19.8
12	8	F	C	.12	17.8	17.8	17.8
13	7	M	W	.12	15.1	15.3	15.2
14	7	M	W	.18	16.2	16.0	16.1
15	7	M	W	.18	16.2	15.3	15.8
16	9	F	W	.18	15.5	15.2	15.4
17	6	M	W	.18	14.6	----	14.6
18	8	F	C	.19	19.6	19.7	19.7
19	6	M	W	.19	16.2	15.4	15.8
20	2	M	C	.19	15.1	----	15.1
21	8	F	C	.20	17.2	17.1	17.2
22	8	F	W	.20	16.7	16.7	16.7
23	6	M	W	.20	16.6	16.8	16.7
24	7	M	W	.20	16.4	16.6	16.5
25	8	F	W	.20	13.6	13.9	13.8
26	8	F	L.C.	.21	19.0	18.2	18.6
27	11	M	W	.21	18.2	18.3	18.3
28	10	F	W	.21	16.8	18.6	17.7
29	5	F	W	.21	16.6	17.6	17.1
30	6	M	W	.21	15.3	15.6	15.5
31	6	M	C	.21	15.1	15.2	15.2
32	10	M	W	.21	14.4	----	14.4
33	3	M	L.C.	.21	12.2	12.0	12.1
34	60	M	W	.22	20.3	20.2	20.3
35	8	F	W	.22	19.0	19.0	19.0
36	9	F	L.C.	.22	15.2	15.3	15.3
37	6	M	C	.22	9.6	9.4	9.5
38	10	F	W	.23	22.3	22.3	22.3
39	8	F	L.C.	.23	20.2	21.1	20.7
40	8	F	C	.23	17.2	17.1	17.2
41	10	F	W	.23	16.1	16.2	16.2
42	8	F	W	.23	15.5	16.6	16.1
43	38	F	C	.23	14.4	13.7	14.1
44	6	M	W	.23	13.2	----	13.2
45	11	F	W	.24	23.5	----	23.5
46	10	F	W	.24	17.9	17.3	17.6
47	39	F	L.C.	.25	19.6	19.9	19.8
48	6	M	C	.25	14.8	14.8	14.8
49	8	F	W	.27	15.0	15.4	15.2
50	8	F	W	.29	16.0	15.9	16.0
51	39	F	L.C.	.29	14.0	14.1	14.1
52	3	F	W	.29	13.8	14.4	14.1
53	6	M	C	.29	10.8	11.1	11.0
54	8	F	C	.30	15.8	16.2	16.0
55	9	F	W	.30	15.9	16.0	16.0
56	10½	F	W	.30	15.4	15.7	15.6
57	7	M	W	.30	15.4	15.5	15.5
58	7	M	W	.30	15.3	15.2	15.3
59	8	F	C	.30	10.6	10.7	10.7
	Mean			.20 ± .07			16.7 ± 2.9

*W.—White
C.—Colored
L.C.—Light-Colored

In another seven instances the skin test times were above 14 minutes associated with a blood level of over 0.7 mg. per 100 c.c. Six of these seven had received ascorbic acid for one to two days preceding the test. There were eighteen other cases where the skin test time was above 14 minutes associated with blood levels between 0.35 and 0.55 mg. per 100 c.c. In all the other cases where the skin test time was less than 14 minutes, the blood levels varied widely and showed no correlation with the skin test times.

Ten children ranging in age from 3 to 11 were placed on a diet which contained no fruits or fruit juices. Their blood vitamin C level and skin test times were done before starting this diet and at weekly intervals while they were on this deprivation diet. The blood vitamin C level fell, and the skin test time became prolonged. It usually took about 9 weeks before the skin test time was over 14 minutes. Two of the children were given ascorbe acid and the skin test time was reduced to normal. A typical case, A. G., is given in detail in Table III. Gross and some biomicroscopic examinations of the gums in these cases revealed many of the gingival manifestations of avitaminosis C which have been so brilliantly described by Kruse.[20] It is to be regretted that photographs of these gums could not be taken regularly during the time the children were on the deprivation diet.

TABLE III

A. G., WHITE BOY ON VITAMIN C DEFICIENT DIET

DATE	SKIN TEST TIMES MINUTES			BLOOD ASCORBIC ACID MG.
	I	*II*	*Average*	
2/11/43	7.3	7.6	7.5	1.33
2/16/43	6.8	6.9	6.9	1.31
2/23/43	7.5	7.6	7.6	1.47
3/ 2/43	9.2	9.4	9.3	0.81
3/ 9/43	8.6	9.2	8.9	0.46
3/16/43	8.8	9.3	9.1	0.51
3/29/43	8.0	8.3	8.2	0.53
4/ 5/43	12.0	12.0	12.0	0.45
4/12/43	15.5	15.4	15.5	0.30
4/19/43	16.4	16.6	16.5	0.20

Forty-five cases were given 200 mg. intravenously of ascorbic acid immediately after a skin test and blood vitamin C were done. Thirty minutes later the skin test and blood vitamin C were repeated. In 44 cases the skin test time was reduced and the blood level of vitamin C was raised.

REACTIONS

The reactions with this skin test have been negligible. Two children developed pinhead-sized red nodules at the site of the injection which persisted for two weeks. These nodules were not associated with pain. A number of patients complained of a burning sensation at the site of injection during the test.

COMMENT

Previous reports condemned the intradermal dye test for two main reasons. First, the test used gave varying times when done repeatedly in an individual.

The method and technique which we have suggested and used obviates this criticism. Second, the skin test did not correspond to the blood vitamin C levels.

There has been a tendency to overestimate the value of the blood level of vitamin C in appraising nutritional status. Kruse[21] states "it should be clear that there is no necessarily high correlation between data derived by different methods on the same deficiency disease. They provide information on different aspects and states of the disorder. Unfortunately, this fact has not been appreciated. Rather, it has been thought that various methods applied to the same deficiency disease should yield similar data. On this basis it has become the practice to test the validity of a method by comparing its results with blood values. This procedure is entirely unsound. When it is remembered that blood values shift rapidly and may fluctuate intermittently, while tissue changes vary slowly, there should be no expectation of identical results. . . . Values on the concentration of a vitamin in the blood reflect very sensitively the recent dietary habit as well as other conditioning factors. They may change not only with season but also within shorter periods; they may fluctuate. . . . Potent therapy will produce maximum blood levels and entirely restore bodily saturation in several weeks, but will completely repair the slightest chronic tissue lesion only in months."

Our results in animals and human beings indicate that the skin test gives information as to the body saturation with vitamin C. The blood level reflects the recent intake of vitamin C. Therefore, the prolonged skin test time of over 14 minutes, which is usually associated with a blood level below 0.3 mg., and gross and biomicroscopic changes in the gums indicate a marked degree of tissue unsaturation. In some patients who took ascorbic acid on the day before the test the blood level was high, but the skin test remained prolonged over 14 minutes because the tissue reservoir was still depleted and had not been replenished.

At present we feel that skin test times of 9 to 13 minutes probably indicate mild degrees of unsaturation, while times of less than 9 minutes indicate satisfactory vitamin C nutrition. Skin test times of 7 minutes or less probably indicate complete saturation.

The blood level, skin test, gross and microscopic examination of the gums all give valuable information in evaluating the nutritional status as to vitamin C. The blood level indicates the recent intake. The skin test indicates the degree of body saturation and unsaturation. The gross and biomicroscopic examination of the gums indicate the changes that have taken place in the tissues. It must be remembered that once tissue changes have taken place, restoration to normal requires some time even though body saturation with vitamin C is maintained.

The skin test is so easy to perform, quick, and cheap that it may find a wide field of application. It can be used as one of the methods in evaluating the nutritional status as to vitamin C in an individual. It could be used in screening school children, hospital admissions, and the armed forces. The fact that the test can be read within twenty minutes makes it very convenient for office practice.

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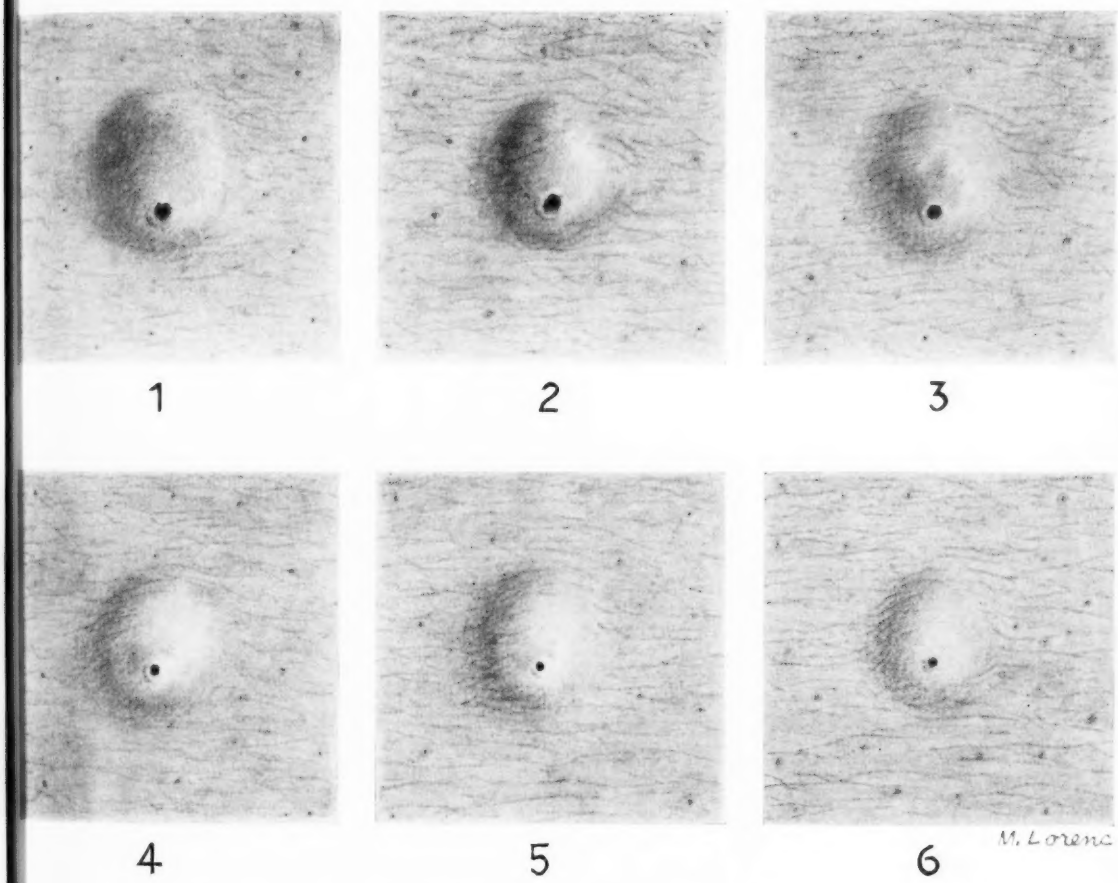

Fig. 2.—Intradermal test in patient with vitamin C subnutrition (painted from life).

1, Immediately after injection; *2*, 4 minutes after injection; *3*, 8 minutes after injection; *4*, 12 minutes after injection; *5*, 16 minutes after injection; and *6*, 17½ minutes after injection, disappearance of all blue color except the pin-point darker blue spot where the needle entered the skin. This pin-point darker blue spot is not to be considered in determining the decolorization time.

The skin test would not take the place of gross and biomicroscopic examination of the gums. However, where gross and biomicroscopic examination have demonstrated tissue changes and the patient is being treated, the skin test could follow the efficacy of therapy. The best therapy is that which produces body saturation. Thus, the dosage of ascorbic acid should be that which will give a skin test time of seven minutes or less constantly.

It may also be possible to work out saturation tests with the skin test. At present the saturation tests are done by following the blood levels and urinary excretions of vitamin C after a test dose. These give valuable information but are time-consuming and difficult to perform. It seems likely that the study of skin test times before and after test doses may yield similar information with less effort and expense. The use of small test doses would be particularly helpful in those patients who had skin test times between nine and thirteen minutes and whom we consider to have mild degrees of unsaturation. When the skin test time is more than fourteen minutes, there is a definite vitamin C subnutrition.

This preliminary report is presented with the hope that others will check our results promptly. The intradermal test for vitamin C subnutrition can be useful during the war and postwar days.

SUMMARY AND CONCLUSIONS

1. Our intradermal test, raising an approximately 4 mm. wheal with a N/300 dichlorphenol indophenol solution, will indicate vitamin C subnutrition states.

2. Of 59 patients with blood vitamin C levels below 0.3 Mg., the skin test times were more than 14 minutes in 54. In 10 children on vitamin C deprivation diets, the blood levels fell, and the skin test times became prolonged. The skin test times were then reduced to normal following administration of ascorbic acid.

3. A skin test time of more than 14 minutes suggests a definite degree of body unsaturation, from 9 to 13 minutes mild unsaturation, and less than 9 minutes a normal amount of vitamin C in the body tissues.

4. Different tests give information on various aspects of the vitamin C nutritional state and thus will not always correlate. The blood level reflects only recent dietary intake. The skin test parallels the degree of body saturation. Gross and biomicroscopic changes in the gums demonstrate actual tissue changes.

I wish to thank Miss Joan Mestern for her valuable aid.

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ON THE PERSISTENCE OF FALSELY POSITIVE SEROLOGIC TESTS FOR SYPHILIS IN NONSYPHILITIC INFECTIONS

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IN THE two decades following the introduction of the Wassermann test in 1904, false positive reactions were reported in a great variety of conditions, notably yaws, infectious mononucleosis, malaria, trypanosomiasis, relapsing fever, leprosy, tuberculosis, diabetes, pregnancy, anesthesia, malignancy, jaundice, fever in itself, and hypercholesterolemia. During the third decade after 1904, increasing refinement in technique, "notably in the direction of less anticomplementary antigens"[9] showed that of all these conditions only yaws gave a uniformly positive reaction, while mononucleosis, malaria, trypanosomiasis, relapsing fever, and leprosy did so occasionally, with a frequency that varied with different observers.[8] Both the Wassermann reaction and the more sensitive precipitation tests began to be considered as "amazingly specific," so that if these six diseases could be excluded, a positive, or certainly a repeatedly positive, Wassermann or Kahn test was taken as definitely indicative of the presence of syphilis.

Of late years it has become increasingly clear that the specificity of these tests was less than had been supposed. An interesting observation was made when the Wassermann and Kahn tests were applied to animals.[6] It was found that rabbit blood in particular but also that of chickens, pigeons, rabbits, mice, cattle, calves, sheep, lambs, horses, and swine frequently gave positive reactions. It is true that the reagin found in the blood of normal animals differs in certain fundamental respects from that of syphilitic blood:[3] it is more labile, is associated only with euglobulin instead of also with pseudoglobulin, and gives a complement fixation test with many lipoids that do not affect human syphilitic sera. In the routine tests, however, normal animal and human syphilitic blood behave exactly alike.[5]

The simplest method of distinguishing between the reagin of animal blood and that of syphilitic sera appears to be Kahn's verification test. Kahn[5] found that with his standard test human syphilitic serum gives a positive reaction at 37° C., but a negative one at 1° C. Animal blood does the reverse, being positive at 1° but negative at 37° C. Strongly positive blood sera of either kind may react at both temperatures; they must then be diluted.

The value of the Kahn verification test as a method of distinguishing between true and false serologic positive is still under discussion, some observers[2, 12] finding it quite trustworthy, while others[10, 13] reporting repeated failures. From the literature, one who has not used it gets the impression that while often useful, it is not always reliable.

Because of the presence in normal animal blood serum of a reagin that gives a positive Wassermann and Kahn test, it is not surprising that a similar

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†Deceased.

reagin should have been found in normal human blood. Malloy and Kahn[7] in 1931, and after them a number of other observers,[1, 14] have shown that normal human sera contain this reagin, but ordinarily in too small an amount to show with our usual tests. Occasionally perhaps a normal human serum may contain enough of this reagin to give a positive test for syphilis. Eagle[4] in 1941 reported serologic studies done on 40,545 white college students of both sexes. In some ten cases (1:4000) a positive serology was found without other evidence of syphilis. Similar findings have been reported by others.[14] The interpretation of these findings is not easy. As Eagle points out, they may be:

1. True false biologic positive reactions
2. Cases after all of latent syphilis
3. Cases of recent intercurrent infection.

Unfortunately they could not be followed up serologically. Statistically the group of false biologic positive reactions is negligible, but in each individual case the interpretation of the reaction may be of the highest importance.

In cases of this kind, the possibility of error in laboratory technique cannot always be excluded. Since such technical lapses do occur, although rarely in the best serologic laboratories, no patient without other evidence of syphilis should be labelled syphilitic on the basis of serology alone until he has received the most careful kind of individual study. The following case may be in point: The patient, a married woman with healthy children, offered herself as a blood donor but was turned down because her Kahn test done twice was strongly positive on each occasion. Almost immediately her physician sent her blood to two other laboratories, by both of which Kahn, Kline, and Wassermann were reported negative. Was this a technical error? Perhaps not. O'Leary[10] speaks of a group of patients "who give biologic or false positive reactions which fluctuate from week to week and from month to month."

A much more important source of error is found in the nonsyphilitic infections that may show a positive serology. The general impression, however, is that these falsely positive Wassermann and precipitation tests are positive "only during the active stage of the disease."[11] That this is not the case I have had an opportunity repeatedly to note. The Kahn test may remain positive for as long as two weeks after defervescence as is illustrated by the following case:

Mrs. T. came to the office complaining of vague malaise, chiefly vertigo with headache, weakness, and a chilly feeling. Temperature, pulse, and respiration were normal, and physical examination was negative; in particular, there was no splenomegaly. The urine contained 3.5 per cent of sugar; the Kahn test was four plus. No blood spreads were made. A few days later a second tube of blood was sent to a private laboratory, and the Wassermann and Kline tests were each reported four plus. The patient and her husband were summoned to the office; the nature of syphilis was explained and prolonged treatment urged. Ten days later she returned to the office stating that she had had a chill two days before and again just before leaving home. Her temperature was found to be 105° F.; again no spleen was felt, but the blood contained many tertian parasites. There was prompt recovery under quinine therapy. Two weeks after defervescence, however, the Kahn test was still positive, though less intensely so than at first. After another two weeks, the test was negative and remained so. Interested by this case, Dr. Orgel and I

analyzed 154 cases of malaria, almost certainly nonsyphilitic, from the wards of the Jewish Hospital and City Hospital and from our own practice. The Kahn test was positive in 22 per cent, and in the cases in which a Wassermann was done, the same percentage of positives was found. What I should like to emphasize particularly, however, is the observation that of 13 cases followed after defervescence, 9 still showed a positive serology eight days after all evidence of fever had ceased, while one was still positive fourteen days after defervescence. All were negative fifteen days after defervescence.

A positive test for syphilis has been repeatedly found in rat-bite fever, but how long it may persist is shown by the following case:

M. L., aged 13, was brought to the Jewish Hospital on Oct. 14, 1934, with high fever and what looked like facial erysipelas. The mother stated that two weeks before admission the child had slept on the floor and had apparently been bitten under the left eye by a rat. The facial lesion had healed, but then had reopened with extensive cellulitis. After admission, she ran a high spiking fever for nearly two weeks. The temperature then became normal, but the lesion on the face did not entirely heal until a short time before her discharge six weeks later. Two mice received each 1 c.c. of the patient's blood intraperitoneally; after fourteen days, smears from one of the mice showed the presence of *Spirillum minus.*

A Kahn test was not done until two weeks after defervescence, when it was found four plus, the Wassermann being negative. The Kahn test was repeated at irregular intervals, thereafter, and was still four plus on Dec. 20, eleven days after she had been discharged clinically well. Unfortunately she then received in the Out-patient Department 5 injections of bismuth sodium tartrate and 3 of neosalvarsan. Following this, the intensity of the Kahn test gradually declined, becoming negative two months later. The Wassermann test remained uniformly negative.

It is of course possible that this little girl really had syphilis. There were, however, no history or physical signs of this disease, and in spite of the grossly insufficient treatment she has shown no evidence of syphilitic infection. She is now married and has given birth to a healthy child.

A more definite case of falsely positive serology was observed at the Jewish Hospital about a year ago.

M. P., aged 36, was admitted to the service of Dr. A. S. Reiches on Dec. 26, 1941, for a cellulitis of the nose following the picking of a nasal hair. The nose was red and swollen to the size of a golf ball. There was no fever, except that the rectal temperature once rose to 100°. Recovery was prompt under hot packs and sulfathiazole, and he was discharged well on Dec. 31. On admission, the Kahn test was found positive, the Wassermann negative; two days later the Kahn test was reported doubtful. In January, however, on two occasions, both Kahn and Wassermann were found definitely positive, only thereupon to become negative and to remain so spontaneously and without any treatment. At no time, then or later, did M. P. give any history of syphilis or show any signs suggestive of this disease. The serology may be summarized as follows:

DATE	KAHN	WASSERMANN	SPINAL FLUID
12/27/41	positive (J. H.)	neg. (J. H.)	
12/29/41	doubtful (J. H.	insuff. (J. H.)	
12/31/41	discharged well		
1/19/42	+++ (Ives)	+++ (Ives)	
1/26/42	+++ (Ives)	++ Kolmer (Ives)	
2/ 2/42	neg. (Ives)	neg. (Ives)	
2/ 9/42	neg. (Ives)	neg. (Ives)	neg. (Ives)
4/ ?/42	neg. (Army board)	neg. (Army board)	
7/ 3/42	neg.	neg.	

Pneumonia has occasionally been found to give a falsely positive serology, and that this reaction may persist for some time after recovery is illustrated by the following case:

Mrs. F. S., aged 40, had long been troubled by chronic arthritis. She went to Excelsior Springs for the baths in 1937; a routine Kahn test taken there was found negative. She bore her first child early in 1941; Dr. Soule informs me that her Wassermann reaction then was negative. Her husband too, when qualifying as a blood donor, was found to have a negative Kahn test. I first saw her on June 2, 1942, for hypertrophic arthritis; a routine Kahn test then was negative.

On June 12, 1942, she entered the Jewish Hospital for an acute respiratory infection that rapidly proceeded to consolidation in the base of the right lung. There were no pneumococci in the sputum; there was a leucopenia and x-ray findings of confluent bronchopneumonia. The final diagnosis was pneumonia, acute interstitial, probably of virus origin. Defervescence took place on June 14, and she felt completely well thereafter. She was discharged on June 19. On the day of admission she showed a four plus Kahn, both Kahn and Wassermann remaining strongly positive for at least nine days after defervescence. She received no treatment, and since June her Kahn and Wassermann tests have been negative. The serology may be summarized as follows:

DATE	KAHN	WASSERMANN
6/12/42	++++ (J. H.)	
6/14/42	defervescence	
6/16/42	++++ (J. H.)	++++ (J. H.)
6/18/42	++++ (J. H.)	++++ (J. H.)
6/19/42	++++ (J. H.)	
discharged		
6/23/42	++++ (Hagebusch)	++ (Hagebusch)
8/24/42	neg. (J. H.)	neg. (J. H.)

The social and medical importance of these falsely positive reactions is much greater than the relative infrequency of their occurrence would indicate. The giving of a Kahn or similar serologic test has become more and more routine, not only in medical practice but in industry, before marriage, in connection with the draft, etc. Among such a huge number of tests many false positives must occur. Many of them are doubtless not recognized as such. The prevalent custom of considering every patient whose blood serum gives a repeatedly positive Kahn or Wassermann test as syphilitic and of immediately inaugurating treatment must mask many such cases. The serology promptly becomes negative and, at the close of a longer or shorter course of treatment, the physician concludes that he has cured his patient even though really the latter may not have been infected at all. A much greater disaster may result from the psychologic shock produced when a young man or woman is mistakenly convicted of being syphilitic, even though later the verdict may be withdrawn.

So far as the physician is concerned, the critical question is whether or not to inaugurate treatment in an individual who lacks history or signs of syphilis but who shows a repeatedly positive serology. It is today a commonplace that insufficient antisyphilitic treatment is worse than none and that one must choose between doing nothing and an adequate course of treatment that may take two years or more to complete. What then should be done with a patient who shows no other evidence of syphilis than a repeatedly positive Kahn or Wassermann or both? The following procedures will probably prevent error in most cases:

1. If the patient has recently had an acute infection of any kind, repeat the Wassermann and a precipitation test in a month or, better, two. If the test is still positive, the reaction is not due to the infection and is probably indicative of syphilis.

2. If a history of recent infection is not obtained, the patient may still be a member of the group described by O'Leary[10] who give "false positive reactions which fluctuate from week to week and from month to month." Here, weekly tests if not constantly positive may reveal the patient as not syphilitic.

3. If spinal puncture is done, its results may settle the matter, since a falsely positive spinal fluid has not as yet been reported.

4. In doubtful cases, Kahn's verification test may be employed, although as yet its results must be interpreted with reserve.

5. Sera that are positive with less sensitive techniques, such as the Wassermann test, but negative with more sensitive ones, such as the Kahn or Kline tests, are probably not syphilitic.[8]

SUMMARY

The occurrence of positive serologic tests for syphilis in the blood of non-syphilitic individuals is discussed. Its occurrence in various infections, not only during the active stage of the disease but for some days or weeks thereafter, is illustrated by four cases. A plea is made not to inaugurate treatment in a patient without other evidence of syphilis than a positive serology until further observation has made it clear that the reaction actually is due to syphilis.

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DEATHS FOLLOWING THE USE OF LOCAL ANESTHETICS IN TRANSCRICOID THERAPY: A CRITICAL EVALUATION

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IT IS the purpose of this paper to emphasize the dangers associated with the use of cocaine and pontocaine when these agents are used in the preparation of patients for transcricoid therapy. A review of the literature on this subject would lead one to believe that fatalities following the use of these analgesics for pharyngeal anesthesia are by no means common. Our attention, however, has been directed to five deaths which have recently occurred in the Charity Hospital of New Orleans following the use of one or both of these drugs. Most of the patients were elderly and were being subjected to the instillation of iodized poppy seed oil for diagnostic purposes (Fig. 1). Of the five patients reported, four died before the instillation of the iodized oil, thus eliminating sensitivity to constituents of the oil, i.e., the iodine, the oil itself, or contaminants.[1] In the fifth instance, iodine sensitivity had been precluded by preliminary testing.

Detailed discussion of these cases serves little useful purpose because of their great similarity. An example which illustrates the sequence of events following the application of local anesthetics is that of a white woman, aged 65, who entered the hospital with chief complaint of pain in the neck and headache. The clinical impression at the time of admisssion was psychoneurosis, possible arteriosclerotic heart disease, possible Ménière's syndrome. During her stay in the hospital, a lipiodol instillation under the fluoroscope was suggested, to rule out bronchiectasis. Starting at 3:10 P.M., the patient's throat was sprayed with 5 per cent cocaine HCl, from a fine atomizer, at intervals of four to five minutes, for five times. She expectorated the excess cocaine. A total of about one cubic centimeter was used. The patient was talking during this time and showed no evidence of toxic reaction.

She walked across the hall to the fluoroscopic room. Under the fluoroscope, a catheter was inserted through the right nostril and into the trachea. The patient tolerated this well. About 4 c.c. of 2 per cent pontocaine were injected into the trachea. She coughed a little and tried to converse. A few seconds later, before the lipiodol was injected, she suddenly became rigid and slid off the stool onto the floor. She had a short clonic convulsion, became cyanotic, and expired. She did not respond to stimulants or to artificial respiration and was pronounced dead at 3:50 P.M. No gross or microscopic anatomical pathologic findings to account for the death were noted. The relevant data of the other four cases are presented in Table I.

*From the Department of Medicine, Tulane University School of Medicine.
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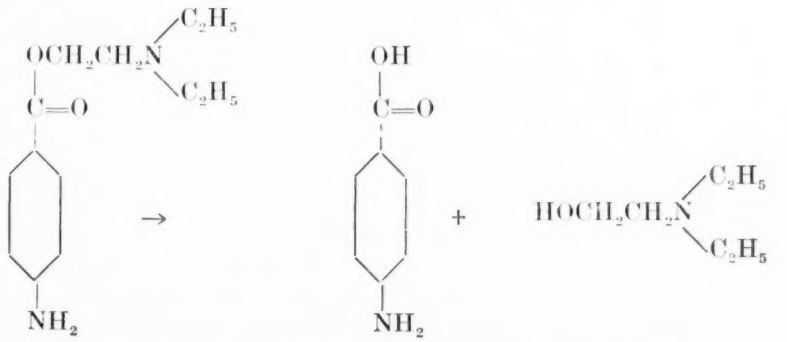
TABLE I

CASE	AGE	SEX	RACE	DRUG	SYMPTOMS	RESULTS	AUTOPSY	MODE OF DEATH
L. S.	65	F.	W.	Cocaine HCl, 5 per cent, 1 c.c. Pontocaine 2 per cent, 4 c.c.	Cough, inability to talk, short clonic convulsions, cyanosis	Death in 40 minutes	Negative	Respiratory failure
W. K.	56	M.	W.	Cocaine 5 per cent spray Pontocaine 2 per cent, several c.c.	Clonic convulsions	Death in 4 minutes	Negative	Respiratory failure
J. M.	72	M.	W.	Pontocaine 2 per cent, 15 c.c. spray	Cyanosis	Death in 6 minutes	Negative	Laryngeal asphyxiation
C. G.	51	F.	W.	Pontocaine 2 per cent, 2 c.c.	Difficult respiration, then apnea	Death in 15 minutes	Negative	Respiratory failure
E. B.	43	F.	C.	Cocaine, or Pontocaine	Frothing from mouth, restlessness	Death in 2 hours	Negative	Respiratory failure

DISCUSSION

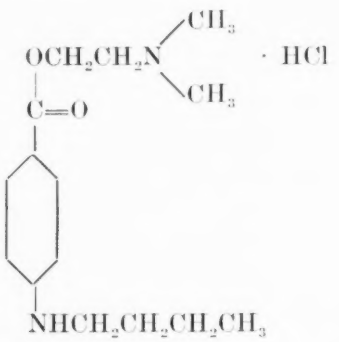
Various local anesthetics are used today because they have the power of blocking locally the transmission of stimuli. Inasmuch as it is necessary to abolish swallowing and gag reflexes, the use of these drugs in transcricoid therapy is indispensable. A variable amount is absorbed depending to a large extent on the vascularity of the anesthetized area. Detoxification follows absorption. Goldberg, Koster, and Warshaw[2] have delineated certain of the steps involved in the detoxification of procaine. They found that procaine may be hydrolyzed at the ester link to form para-aminobenzoic acid and diethylaminoethanol, a reaction which takes place slowly in pure water and may be accelerated by catalysts.



Procaine *Paraminobenzoic acid* *Diethylaminoethanol*

Para-aminobenzoic acid has been extensively studied by a number of workers because of its intimate relationship with the sulfonamides. As in the case of these latter drugs, it is acetylated to form para-acetaminobenzoic acid. Many believe that the detoxification of these various substances takes place in the liver, but certain investigators[2] consider that at least a fraction is rendered inert in the blood stream. Goodman and Gilman[3] point out that the liver is capable of detoxifying one minimum lethal dose of cocaine in sixty minutes.

There are few detailed studies on the fate of pontocaine (parabutylamino-benzoyl dimethylamino-ethanol hydrochloride) whose base belongs to the procaine group.



Pontocaine

It is logical to assume, in view of this similarity, that a similar sequence occurs when it is detoxified.

Gilman[4] divides the reactions following the use of novocain into three main types: His first and most frequent is characterized by marked excitement, delirium and convulsions, and respiratory failure. This reaction begins within a variable period after administration of the drug and is frequently of long duration. His second type appears suddenly and, in contradistinction to the first type, is of short duration. In severe cases there is a pallor, fainting, and stoppage of the heart with resultant death. His third type of dangerous reaction is allergic shock. Waldbott[5] has reported urticaria, vasomotor rhinitis, and asthma from nupercaine, cocaine and similar anesthetics. Vaughan[6] has introduced a useful concept to indicate an increased sensitivity or reaction capacity to a foreign substance; namely, hyperergy. Hyperergic individuals react in a normal manner but react excessively. Vaughan illustrates this phenomenon by citing the person who sunburns extremely easily as being hyperergic to the actinic rays; another person who reacts with urticaria after similar exposure is allergic. It seems to us that the reactions following the administration of local anesthetics may be properly divided into two groups. Thus Gilman's first two groups are hyperergic; his first representing primarily nervous system involvement and his second representing primarily cardiovascular involvement.

With regard to Gilman's third or shock group, two possibilities exist. The first is that allergy is not a factor in the production of these reactions. It will be remembered that the allergic reaction is predicated upon the interaction of an antigen and the newly formed immune substance (probably globulin in nature) which has been synthesized stereochemically under the aegis of the sensitizing agent. By its very nature this process is time-consuming, requiring from five to seven days in experimental animals, and varying in the human being from this period to two years or longer.[7, 8] Since these reactions occur on the first exposure, no time has elapsed for antibody formation to occur. It must be emphasized that a latent period between the first exposure to an antigen and the following one must exist for an individual to react allergically. It must

also be borne in mind that the allergic reaction is usually initiated by a substance which is not primarily harmful; hence no ill effects are noted on the first administration.

The alternate hypothesis is that the individual has been sensitized by a previous exposure to a chemically related substance. Examples of naturally occurring substances related to these anesthetics would include the phenolic essential amino acids such as phenylalanine and tyrosine and para-aminobenzoic acid which is a component of the B group of vitamins. It is believed that the allergic reactions to drugs are mediated through linkage substances (Landsteiner) whose identity is unknown. It has been shown that many substances containing the benzene ring may be antigenic. Therefore the allergic hypothesis can only be satisfied if we assume a previous sensitization to a phenolic compound or even to para-aminobenzoic acid, a substance which is widely distributed throughout nature.

Thomas and Fenton[9] submitted samples of the pontocaine solution used in the three deaths they observed, to the Committee on Pharmacology and Chemistry of the American Medical Association. These were reported to contain unmodified, pure pontocaine and nothing else; it was concluded therefore that the fatal reactions were due to drug sensitivity and not to impurities.

Would it have been possible to have foretold sensitivity to pontocaine by the employment of appropriate measures? It is unfortunate that skin tests with drugs, which would be of great aid in helping avoid these reactions, are of questionable value. While patch tests to drugs causing contact dermatitis are highly specific and reliable, drugs which cause trouble following parenteral administration often cannot be satisfactorily tested either by patch test, scratch test, or intracutaneous test.

TREATMENT

The desideratum is for an anesthetic which combines long-acting and adequate anesthesia with no detrimental side effects. Up to the present, no drug meets all of these qualifications.

Furthermore, safe dosage levels have not been established for any of these agents. There is no such thing as a truly safe dose. A quantum can be established which can be tolerated by the vast majority of individuals, but no matter how small the dose used, there are persons who will succumb to it. This statement applies to all of these drugs, for Sollman[10] has indicated that in all probability all locally used anesthetics have produced fatal accidents.

In an attempt to attain the irreducible minimum of fatalities, the Local Anesthetic Committee of the American Medical Association[11] has made certain recommendations. They found that procaine was the safest of the local anesthetics and only if the concentration did not exceed one per cent. The total amount of cocaine should not exceed 0.06 to 0.1 Gm. (1 to 1½ gr.), but deaths have been reported from 0.02 Gm. (⅓ grain) of cocaine. It must be borne in mind that certain individuals have tolerated many times this amount. Of great importance is the site of administration. Fatalities are especially frequent when the urethra, scrotum, and tonsils are anesthetized.

Because it is known that the barbiturates have some slight antidotal effect, preoperative use of these sedatives is advisable. Fussgänger and Schaumann[12]

have shown that the addition of one drop of one-to-one-thousand epinephrine solution to 10 c.c. of 5 per cent pontocaine solution allows the operator to use twice the lethal dose. The epinephrine is added, not to protract the duration of anesthesia, but to decrease the rate of drug absorption. Provided the body's defenses are not immediately overwhelmed, the liver is capable of detoxifying one lethal dose of cocaine in an hour.

It is to be regretted that treatment of the severe reactions reported is of little value. The following procedures have been recommended, although admittedly little is to be gained from their use. Attempts to sustain heart action such as cardiac massage and intracardiac injection of epinephrine have been advocated. Artificial respiration rarely carries the patient through the crisis. When convulsions supervene, some sedative such as sodium amytal may be used.

SUMMARY

A series of five patients dying following the application of local anesthetics incidental to transcricoid therapy is reported. An attempt is made to explain the basis for these fatal reactions in the light of recent studies, and a rational classification of these reactions is presented. Prophylaxis and therapy are outlined.

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THE ROLE OF CREATINE IN CELL GROWTH IN VITRO AND ITS USE IN WOUND HEALING

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PRELIMINARY REPORT

CARREL[1] observed that extracts of muscle and gland tissues of adult animals stimulate the rate of growth of homologous fibroblasts in vitro. Carrel and Ebeling[2] demonstrated that chicken fibroblasts could be made to live and multiply indefinitely in vitro when the medium contained small amounts of embryonic tissue juice. They suggested that this proliferation is probably occasioned by certain nitrogenous compounds.

Recently Doljanski and co-workers[3] have extended these experiments to include the growth in tissue culture of human skin epithelium. They found a Tyrode extract of the heart muscle of various species not only strongly stimulated the growth of chicken cells but human skin epithelium as well, and they proposed the application of these extracts for wound healing.

The application of heterogenous extracts which contain various unidentified chemical entities, some inert substances, and possibly some inhibitors affords the scientific worker merely an empiric tool with which to initiate more fundamental studies. Indications of the wide field of application for tissue extracts make the identification of the substance or substances which manifest this cell activating and proliferating property one of paramount significance. The disclosure of an active substance will readily make available a definite class of substances for further study, will provide an opportunity to evaluate the specificity of a pure substance in relation to its cell growth-promoting and healing properties, and it may shed light upon other physiologic processes hitherto considered obscure.

This preliminary report is submitted to indicate that a successful attempt has been made to identify at least one of the active substances. A more comprehensive report will be detailed in the near future. Various tissue extracts, heart muscle extracts, and embryonic juice were found by chemical analysis to contain significant amounts of creatine and phosphagen.

Creatine was tested on the rate of growth of a variety of tissue cultures made through the courtesy of Miss Gladys Cameron in Professor Robert Chamber's Laboratory at Washington Square College, New York University. The results were found to be as follows: Tyrode solutions containing 400 to 800 mg. of creatine per 100 c.c. stimulate the growth of adult mouse bladder epithelium, adult mouse fibroblasts, human adult connective tissue and fibroblasts. Tyrode solutions containing as little as 100 to 200 mg. of creatine per 100 c.c. stimulate the growth of chick embryo fibroblasts, rat kidney and liver epithelium.

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A simple linear relationship exists between the creatine-induced area outgrowth of all cultures and the untreated tissue controls. This ratio is calculated by measuring the radii of the outgrowth areas. The mean radius of these measurements is compared with mean radius of the control. The areas represented by these radii will vary as the squares of their corresponding radii. The area ratios do not reveal the thickness and density of these outgrowths. In all the creatine-treated cultures the growth was thicker and more densely packed. In Table I are recorded a few typical results and the corresponding number of experiments performed to determine the activity of the creatine with respect to a specific tissue.

TABLE I

THE EFFECT OF CREATINE UPON VARIOUS TISSUES

TISSUE	DAYS' GROWTH	NO. OF EXPERIMENTS	RATIO OF INDUCED OUTGROWTH AREA TO CONTROL AREA
Chick Fibroblasts	9	11	16:1
Mouse Bladder	6	16	6:1
Human Connective Tissue	10	10	4:1
Rat Liver	6	14	22:1
Rat Kidney	6	14	5:1

May we consider creatine and possibly other guanido amino acids as protoplasmic precursors? Clinical work is still in progress to determine the influence of the injections of solutions of various guanido amino acids upon the healing of internal ulcers. At present, these results are encouraging.

Sandweiss and co-workers[4] observed that pregnancy has a beneficial effect on the symptoms of peptic ulcer, and they focused their attention upon the anterior pituitary-like hormone.

Experimental ulcers were produced in dogs by the Mann-Williamson operation of surgical duodenal drainage. These Mann-Williamson dogs were given daily injections of varying doses of antuitrin-S. Because of this treatment 50 per cent had no ulcer and 20 per cent had ulcers in process of healing. Of the control Mann-Williamson dogs, 98 per cent died with jejunal ulcers. Patients suffering from active symptoms of peptic ulcer responded to antuitrin-S injections in a manner comparable to the Mann-Williamson dogs.

These experimental results provoked our interest in the possible effect of antuitrin-S upon growth of tissue cultures (in vitro). Accordingly, antuitrin-S powder* was dissolved in Tyrode solution to yield a concentration of 400 I.U./c.c. The cell-proliferating property of this preparation was entirely negative for embryonic as well as for adult tissue. The tissues tested were human connective tissue, chick embryo fibroblasts, and epithelium and kidney tubules. From these results it is apparent that the beneficial effects of this hormone are not due to any direct effect upon ulcerated tissue, and we must search for an explanation of this healing mechanism.

Our disclosure of the value of creatine in cell proliferation and wound healing affords us a plausible explanation of the modus operandi of the anterior pituitary-like hormone.

*Dr. Oliver Kamm of the Parke, Davis & Co. generously furnished us with the antuitrin-S powder.

It is known that the anterior pituitary hormones affect creatine metabolism, and the following three references selected from many others will tend to explain why pregnancy has a beneficial effect upon symptoms of peptic ulcer, and how injections of antuitrin-S may beneficially affect peptic ulcers.

Schrire and Zwarenstein[5] showed that during pregnancy the anterior pituitary gland hypertrophies increasing the formation of creatine. Gorostiaga[6] demonstrated that blood creatine during pregnancy and childbirth is increased 7.1 to 7.6 mg. per 100 c.c. Shapiro and Zwarenstein[7] studied the South African toad and noted 15 per cent decrease in muscle creatine following hypophysectomy. Daily injections of anterior lobe extract produced an increase of 30 per cent in muscle creatine.

Daily injections of anterior pituitary hormone increase the formation of creatine, its retention in various tissues, and its concentration in the blood. On the basis of our disclosure of activity of creatine as wound healer we conclude that the similar activity of anterior pituitary hormone in vivo is in part at least due to its effect upon creatine metabolism.

The author is indebted to Dr. Frances Krasnow for her cooperation in this study.

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STUDIES IN COMBINED DIURETIC THERAPY*

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INTRODUCTION

IT IS well known that digitalis becomes finally ineffective in perhaps one-half the cases of congestive heart failure. It is least effective and most toxic in the most advanced forms. Since Jendrassik's introduction of calomel in the therapy of edemas, in Wagener's clinic in Budapest, from 1892 on, the search for more potent and relatively nontoxic diuretics has continued. Guy's hospital pill incorporated 1 grain each of calomel, digitalis, and squill. Merbaphen or novasurol, the first organic mercurial introduced by Saxl and Heilig in 1920, was too toxic and was soon superseded by mersalyl or salyrgan. At present we have the clinical choice of mercupurin, salyrgan-theophylline, esidrone, and some less well-known organic mercury compounds made in 10 and 13.5 per cent solution of the mercurial salt for intravenous or intramuscular use. Most of them now contain theophylline to the extent of some 5 per cent of the solution. The combination is more easily absorbed[1] and less irritating and destructive to the tissues. This may be due to the addition of the somewhat acidic theophylline to the corrosive alkaline hydrolytic products of the mercurials. Mercupurin was thus shown to be much less irritant locally than the original mersalyl by DeGraff and Batterman in 1935.

With the introduction of acid-forming salts, urea, hypertonic glucose, and the xanthine group as additional diuretics, the idea of combining them for additive and possibly synergistic effects arose. This was fostered by the elucidation of their varied modes of action, such as the production of urea and acidosis in the case of ammonium chloride and the direct renal effects of mercurials. Thus Keith and Whelan[2] found that ammonium chloride and novasurol produced greater diuresis than either alone, and together they were effective in cases that were otherwise refractory.

Since 1931, while Schmitz[3] was working with dogs, the Texas group[4-6] has studied the effects on patients in congestive failure with edema, attempting to prove the idea that the xanthines act primarily by increasing glomerular filtration, and the mercurials by blocking tubular reabsorption of fluid. Some have dissented.[7] Through the study of renal clearances of creatinine, d-xylose, urea, and more recently of diodrast and inulin, these ideas have been and are being put to the test. In an early paper[4] in 1933 from this Cardiovascular Service this statement was made: "These observations and facts suggested a possible complementary therapeutic combination of the two modes of action by a proper sequential or simultaneous administration of the two types of diuretics." Theophylline ethylenediamine was administered by mouth or in-

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travenously together with intravenous salyrgan, and increases of urinary output of 100 to 400 per cent were noted. In one case an increase of 840 per cent was noted in the case of a patient who had received 0.48 Gm. of theophylline ethylenediamine intravenously, followed in a few hours by 0.1 Gm. (1 c.c.) of mersalyl by vein. The "oral purine-intravenous mercury" regime as well as other combination regimes have been advocated in previous publications from this institution.[8-9]

On the wards of John Sealy Hospital and in the Cardiac Clinic, our custom in patients needing diuretics in addition to digitalis is to give a two- or three-day course of oral ammonium chloride (up to 6 or more Gm. per day) or theophylline ethylenediamine (up to 1 Gm. per day). This is followed by the intravenous administration of mersalyl-theophylline or mercupurin. This is the classical method. In some cases of gastrointestinal intolerance, the use of a single dose of potassium or ammonium chloride or nitrate (2 Gm.) or theophylline ethylenediamine (0.4 Gm.) some two hours before the mercurial has been found to be as effective. This is the British method of Evans. In the most intractable cases of edema, theophylline ethylenediamine (0.48 Gm.), or a substitute, and the mercurial (2 c.c.) are given intravenously in the same syringe.

The results of diuretic therapy in the John Sealy Hospital were summarized,[8] and it was noted that decholin alone was relatively ineffective, but when injected intravenously (10 c.c. of 20 per cent) together with a mercurial a conspicuous diuresis resulted. Decholin also seemed to be particularly effective in diminishing liver engorgement.

Goodman and his co-workers[10] have reported a series of cases in which an accidental intravenous injection of 7½ grains of theophylline ethylenediamine one hour before intramuscular mersalyl (1 c.c.) started the reinvestigation of combined diuresis. They claim that the particular combination mentioned is the most effective. On the other hand, they claimed to have obtained greater diuresis from mersalyl alone than from mersalyl and aminophylline given simultaneously. Their explanation of the relative failure of the latter combination, as being due to quicker absorption and excretion of the mercurial, does not seem convincing. The same argument is used to prove the superiority of salyrgan-theophylline to salyrgan. Their study relied on daily weights only. We, on the other hand, by comparative studies in the same patient and in different patients, believe we have shown that some other combinations of intravenous diuretics are the most potent yet devised. At least, such combinations inaugurated brisk diuresis in cases which had proved refractory to all other methods.

METHOD

The patients used in this study were typically chronic cardiacs in congestive heart failure unrelieved by bed rest and digitalis. They were continued on this regime, the usual low salt cardiac soft diet, an intake of approximately 1500 c.c. of fluids daily, and occasional oral acid salts or decholin. The diuretics were used in varying rotation. Mercurials in 2 c.c. dosage, theophylline ethylenediamine 0.48 Gm., and the two combined were so used intravenously.

In a few cases theophylline methyl-glucamine (glucophylline, Abbott), theophylline isobutanolamine (Merrell), theophylline isopropanolamine (National Drug Company), theophylline sodium acetate (Upjohn), and decholin (Riedel-de-Haen) were used in 10 c.c. dosage instead of aminophylline. In four cases aminophylline intramuscularly was injected two hours before the mercurial. Daily weights were unfortunately not obtained in all cases, for one reason, because of the absence of a scale capable of weighing bedridden patients. Because of that, comparative diuresis statistics are based on three-day percentage increases of urinary output, as in previous studies from this institution.[8] Ideally, of course, a weighed diet containing a known constant liquid and salt content, a bed scale capable of weighing to fractions of a pound, and more exact measurements of fluid output, including the amount lost in the stools, would be desirable.

It is worth mentioning that, while comparable statistics relating to each drug or combination are significant if enough cases are available, their comparison in individual patients is, perhaps, more significant. One drug or combination may fail in one patient and not in another because of individual differences in degrees of edema, digitalization, hypoproteinemia, cardiac decompensation, liver and kidney damage, and the like. For this reason the study of one patient, such as R. M. S. (see below), for several months, is quite rewarding.

RESULTS

Table I summarizes the diuretic effects obtained in 94 trials in 16 patients. It may be profitably compared with Table II (Drs. Herrmann, Decherd and Associates' 1940 series[8]). It will be seen, that, in general, mersalyl, fortified with xanthines or decholin, caused a greater diuresis than mercupurin or mersalyl-theophylline alone. The combination effects were at least additive, if not synergistic. Certain discrepancies are immediately discernible. The new smaller series show much lower values for diuresis in almost all subdivisions. It must be remembered, however, that for the sake of studying individual refractory edema cases, the cases chosen would show lower diuresis figures. In many of them mercurials alone caused hardly any diuresis; and that was even more true of the xanthines and decholin. The four cases receiving aminophylline intramuscularly prior to mercurials intravenously are too few to have significant statistical value. They are not strictly comparable to the cases in Goodman's series, since the order of administration in their cases was reversed.

The mercupurin combination responses are, in general, disappointing, except, possibly, for the theophylline sodium acetate combination. In the older series, too, mercupurin and aminophylline gave statistically lower diuretic responses than mercupurin alone. However, in the older experimental work[6] and in the new clinical series, the immediate diuresis (note first day figures) seemed to be increased by the combination. Moreover, in individual cases the combination responses were more striking than the figures would indicate. Against the validity of the statistics as a whole may be levelled the criticism that the probable error is high, due to extreme variations of response to the same drug or combination in the same patient and in different patients.

TABLE I

SUMMARY OF DIURETIC EFFECTS

DRUG	NO. CASES	% AVERAGE OUTPUT URINE INCREASE			
		1ST DAY	2ND DAY	3RD DAY	TOTAL 3 DAYS
Mercupurin	27	292	57	46	395
Mersalyl-theophylline	15	237	18	23	278
Theophylline-ethylenediamine	8	91	109	61	261
Mercupurin + theophylline ethylenediamine	8	347	10	1	358
Mercupurin + theophylline sod. acetate	5	628	38	69	735
Mercupurin + glucophylline	2	330	0	20	350
Mercupurin + decholin	3	310	75	0	385
Aminoph. intramusc. then mercupurin intraven.	2	211	377	40	628
Mersalyl-theophylline + theophylline ethylenediamine	8	461	31	29	521
Mersalyl-theophylline + theophylline isobutanolamine	5	277	0	31	308
Mersalyl-theophylline + glucophylline	5	438	20	8	466
Mersalyl-theophylline + decholin	4	479	42	18	546
Aminophylline intramusc. then mersalyl intraven.	2	425	18	5	448

TABLE II

SUMMARY OF DIURETIC EFFECTS

(Drs. Herrmann, Decherd and Associates' 1940 series)

DRUG	NO. CASES	% AVERAGE OUTPUT URINE INCREASE			
		1ST DAY	2ND DAY	3RD DAY	TOTAL 3 DAYS
Mercupurin	611	477	152	79	707
Salyrgan	125	341	120	62	526
Salyrgan and theophylline	75	335	107	42	484
Mercupurin and aminophylline	10	415	154	33	562
Aminophylline	29	263	119	54	436
Theophylline sod. acetate	6	30	59	26	115
Decholin	22	83	34	11	128
Decholin and mercupurin	7	770	101	7	878

As regards individual patients, combined diuretic therapy in all forms was more effective than mercurials alone in 11 out of 16 patients, questionably more effective in two more, and as effective in the other three. No toxic effects were noted except in one patient, who had reactions to either mercupurin or aminophylline. The same patient tolerated mersalyl-theophylline well. Two cases may be singled out for demonstration.

CASE 1.—R. M. S., John Sealy Hospital No. 78659, was a 31-year-old rheumatic cardiac patient, with trivalvular stenosis, auricular fibrillation, cardiac cirrhosis, and ascites, who had been studied over four months. On digitalis, decholin, and acid salts by mouth he failed to respond to repeated mercurial and xanthine injections. After hospital admission, on intravenous salyrgan-theophylline plus glucophylline he showed a marked diuresis, whereas the two separately had failed. The replacement of glucophylline by theophylline isobutanolamine resulted in a temporary increase in diuresis. Similarly, the substitution of decholin and later of intramuscular and intravenous aminophylline resulted in greater diuresis. Mercupurin, likewise, while ineffective alone, caused marked diuresis when combined with theophylline ethylene diamine. In his case, at least, the rotation of diuretics and of their modes of administration appeared to be of therapeutic benefit (see Table III).

CASE 2.—R. G., John Sealy Hospital No. 59192, a 14-year-old Negro boy with constrictive pericarditis, probably of tuberculous etiology, presented marked ascites and gen-

eralized edema. Mercupurin and salyrgan-theophylline in 2 c.c. doses intravenously were at first effective, producing 24-hour urinary outputs up to 4000 to 5000 c.c. More recently, however, the diuretic responses were much lower. Aminophylline alone, in half gram doses intravenously, which had previously produced increased urinary outputs, roughly one-half those following mercurials, now failed completely. Mercupurin or salyrgan-theophylline at times caused no diuresis, and at other times, 24-hour urinary outputs up to 3000 c.c. The mercurials combined with aminophylline gave consistently higher results, with outputs in next 24 hours up to 4500 c.c. The same combined injections kept him in relative comfort in the Out-Patient Clinic, thereby avoiding frequent abdominal paracenteses.

TABLE III

COMPARATIVE DIURESIS WITH MERCURIAL, XANTHINE, DECHOLIN AND COMBINATION DIURETICS IN CASE R. M. S.

DATE	DRUG I.V.	AMT.	% AVERAGE URINE OUTPUT INCREASE				
			1ST DAY	2ND DAY	3RD DAY	TOTAL 3 DAYS	WEIGHT LOSS
11/ 1/42	Mercupurin	2 c.c.	25	0	0	25	0
11/ 5/42	Salyrgan-theophylline	2 c.c.	30	40	30	100	-
11/12/42	Salyrgan-theophylline	2 c.c.	60	60	30	150	0
11/15/42	Glucophylline	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	300	0	0	300	8
11/20/42	Salyrgan-theophylline	2 c.c.	15	0	0	15	0
11/23/42	Glucophylline	10 c.c.	75	0	0	75	0
11/26/42	Glucophylline	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	300	100	0	400	-
12/ 1/42	Glucophylline	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	550	0	0	550	6
12/ 7/42	Glucophylline	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	600	0	0	600	4
12/10/42	Theophylline-isobutanolamine	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	650	0	0	650	5
12/16/42	Theophylline-isobutanolamine	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	250	0	0	250	0
12/21/42	Same as 12/16/42		220	0	0	220	0
12/27/42	Decholin	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	675	0	0	675	2
12/31/42	Same as 12/27/42		720	60	0	780	2
1/ 4/43	Decholin	10 c.c.	75	0	0	75	0
1/ 8/43	Decholin	10 c.c.					
	+ Salyrgan-theophylline	2 c.c.	475	40	0	515	2
1/15/43	Salyrgan-theophylline	2 c.c.	260	0	0	260	0
1/29/43	Aminophylline	0.48 Gm.					
	+ Salyrgan-theophylline	2 c.c.	510	0	0	510	2
2/23/43	Same as 1/29/43		675	0	0	675	3
3/ 3/43	Aminophylline i.m.	0.48 Gm.					
	+ Salyrg-theophyll. i.v. in 1 hour	2 c.c.	700	0	0	700	-
3/16/43	Same as 3/3/43		740	0	0	740	0

DISCUSSION

We may, we think, draw the preliminary conclusions that fortification of mersalyl, with theophylline ethylenediamine up to 0.5 Gm. of the latter results in greater diuresis than the addition of merely 0.1 Gm. of the xanthine. The latter is, of course, the amount in ordinary mercupurin or salyrgan-theophylline. These have already been proved superior to their predecessors, the mercurials without any theophylline added. In a study of creatinine clearances in 1937, Drs. Hermann and Decherd[6] mentioned that "in three instances in which mercupurin was fortified with aminophylline (0.24 Gm.) there was often a

very temporary decrease of filtration before diuresis began, but this was followed by a conspicuous rise in the glomerular filtration along with the characteristic mercurial impairment of tubular absorption. This fortified combination produced a more striking diuresis.

Studies are now being conducted, utilizing diodrast and inulin renal clearances, to measure renal blood flow and glomerular filtration, to study further to see if mercurials and xanthines have different renal loci of action. It has already been shown in previous blood volume studies of Drs. Decherd and Calvin and Herrmann[11] that while the mercurials cause mainly a decrease in blood volume by their renal effect, the xanthines may, after an initial drop, raise the blood volume conspicuously, in spite of any diuresis produced. This may be a possible extrarenal effect of the latter.

SUMMARY

The combinations advocated, then, are the logical outgrowth of past experience and should prove according to previous experimental evidence to be more effective as well as less toxic locally in and about the blood vessels and possibly on the renal tubular epithelium and the liver cell and on all tissues. It has been shown that xanthines protect somewhat against poisoning by organic mercurials.[12] The specific protection, if any, of the liver and kidney needs further proof.

The diminution of liver damage and congestion by decholin is still mainly a clinical surmise and should be further tested experimentally. It is suspected that the choleretic action of decholin and increased circulation in the liver promote the excretion of mercury and fluid from the liver.[13]

Blood volume and renal clearance studies should be of help in the study of the mechanism of decholin diuresis. More clinical data are also needed.

Overdehydration, which may occasionally be accomplished by these powerful diuretic combinations, can certainly cause gastrointestinal disturbances, perhaps muscle cramps, and other so-called toxic manifestations seen after mercurials.

The most that can be claimed for any diuretic therapy is not prolongation of life, but relative well-being of the patient. The question of whether myocardial or renal damage is contributed to by repeated mercurial injections is not yet answered with finality. Theoretically, at least, the heart burden is lessened and digitalis action promoted by the diminution of refractory anasarcas.

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HUMAN SERUM TREATMENT OF ATYPICAL PNEUMONIA

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THE fall and winter of 1942-1943 were characterized by the expected, or somewhat higher than expected, incidence of pneumonia among the personnel of the Glenview Naval Air Station. The disease occurred in both flying and nonflying personnel, with a higher incidence in the former. The usual typical pneumococcus lobar pneumonia was the exception rather than the rule. From Sept. 1, 1942, to May 1, 1943, there were 19 cases of lobar pneumonia and 61 cases of pneumonia of an atypical character, having all the aspects of a clear-cut symptom complex, which has already been described by a number of authors.[1-5] The U. S. Navy has recognized and identified this syndrome in its official nomenclature as "pneumonia, primary atypical, etiology unknown." Other diagnostic terms include "virus pneumonia," "atypical pneumonitis," "acute atypical bronchopneumonia," etc.

The clinical aspects of the syndrome are fairly characteristic; they are, in brief, an illness moderate in severity with gradual onset following a mild upper respiratory infection, severe malaise, nonproductive irritative cough, headache, chilliness, fever rarely exceeding 103° F., a relative bradycardia, normal to very slightly accelerated respiration, minimal or absent physical signs in the chest, a relative leucopenia, a few or no pneumococci in the sputum, characteristic roentgenographic findings diagnostic in themselves, and a negative response to sulfonamides or any other specific therapy for pneumonia.

Our first cases occurred in November and December of 1942. In approximately 65 per cent of the cases the pathology was in the lower lobes, and in over half of these in the right lower lobe. The fever usually fluctuated daily between 100° and 103° F. with a morning peak in more than one-half the cases. This was so characteristic that whenever a man with no physical signs had a fever peak in the forenoon and approached a normal temperature in the evening, we promptly x-rayed his lung fields, anticipating pulmonary infiltration of an atypical character. Chest pain was unusual in our cases. An occasional patient produced blood-tinged sputum. In no cases was there a true chill.

The febrile state lasted from five to eleven days, averaged about six days, and terminated by lysis. The roentgenographic findings persisted long after the fever subsided in all cases, and took upwards of four weeks to subside, occasionally longer. A surprising feature was the prolonged debilitation following a relatively mild disease state. Inasmuch as patients in military hospitals remain hospitalized until ready to return to active duty, there are more sick days for any given condition in military life than in civilian life. We

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found it necessary to keep our convalescent patients as long as fifty days before returning them to duty. This was, of course, particularly true of flight personnel.

The disease has been frequently reported to be of a highly contagious nature. No effort was made to segregate such patients in our wards, and no spread in the ward was observed.

Although fatal cases have been reported,[1] the prognosis in general is excellent. In our series there were no deaths directly due to pulmonary pathology, but one death occurred because of a complicating meningo-encephalitis (confirmed by autopsy). The only other complication we observed was an apparent relapse with a return of the nonproductive cough as the most severe symptom. However, we soon came to believe that this was really due to too early release to active duty.

Treatment with sulfonamides had no favorable effect on the course of the disease, and at times was actually a drawback. Many patients were made more uncomfortable because of nausea, and an already low leucocyte count was sometimes reduced to a dangerous level; accordingly these drugs were discontinued after the first few cases. Oxygen did not seem necessary and was used only once; in the fatal case wherein there was a marked cyanosis, the consolidation involving many lobes.

The only successful treatment so far described from the standpoint of shortening the number of sick days is the use of roentgen therapy. In 95.6 per cent of the author's cases so treated, the febrile period, total number of sick days, and the days for resolution, as proved by x-ray, were reduced roughly one-half.[6]

Wolf and Levinson[7] have reviewed the field of specific human convalescent and hyperimmune sera. They emphasized that "immunotransfusions should be reserved for diseases in which humoral immunity is a significant factor." However, they further state that "the persistence of circulating antibodies years after recovery from an attack of certain diseases is a noteworthy occurrence," and also point out that "normal adult serum provides an appreciable degree of protection against a variety of common diseases."

The natural thought occurred, therefore, that a specific humoral immunity might be set up, if only for a short time, in the symptom-complex described in this paper. Accordingly, patients were chosen from our first "wave" of cases as donors for a supply of serum. Two hundred and fifty cubic centimeters of blood were drawn from each man, no sooner than thirty days after the cessation of fever, and as soon thereafter as possible. This blood was then processed into serum, pooled, and bottled in 250 c.c. units.* This was stored in a refrigerator to conserve antibody content. Only eleven such units were obtained. The small supply was due to the fact that many of our patients are rapidly lost to us through transfer to other places of duty.

Results.—Ten patients were treated with this first supply of serum. We selected an arbitrary initial dose of one unit (250 c.c.). In nine of the cases one unit was adequate to produce a satisfactory clinical response. In only one case was a second unit administered. The latter case will be described in detail,

*The serum was processed by the Samuel Deutsch Serum Center, Michael Reese Hospital, Chicago, Ill. We wish to express our thanks to the director, Dr. S. O. Levinson, for the above, for his generosity in making available to us his wide experience with serum and plasma, and for his constructive interest and aid in this piece of work.

and any one of the other nine will serve for reporting, so typical was the response.

The immediate and late results both were most gratifying. In nine of the patients the fever fell to normal by crisis within twelve to eighteen hours and remained there. No unfavorable reactions were noted. Recovery from the acute symptoms followed rapidly with a moderate cough persisting for from ten to fourteen days. Subjectively, and compared to the untreated cases, the response was good; the patients felt well, wouldn't believe that they had pneumonia, and universally expressed a desire for early return to duty. This latter feeling stood out in marked contrast to the untreated patients who felt exhausted, and showed no inclination to return to duty for some weeks after their fever had subsided. The number of sick days in the untreated cases averaged approximately 39 days; that in the small number of treated patients, 22 days. This accounted for a saving of sick time of approximately 45 per cent.

The x-ray findings as a general rule were not so quickly affected. In several cases, treated within 24 hours of the onset, the consolidation seemed to resolve, as shown by x-ray, within the week. However, in the remainder of the cases, the findings on x-ray disappeared gradually over a period of two weeks. This was somewhat faster than in the untreated cases in which the x-rays were positive for from three to four weeks. However, early resolution was seen in an occasional untreated case, so these results are not considered conclusive.

CASE 1 (Control, Illustrative Case).—J. H. was admitted on 12/28/42 with complaints of chilliness, malaise, headache, sore throat, and cough. Admission temperature was 103.8° F., pulse 94, respiration 24. The physical examination revealed only an injected pharynx. X-ray disclosed an atypical consolidation in the left lower lobe. The leucocyte count was 6,200, with a normal differential. This patient received only symptomatic treatment. Fever subsided on the ninth day (Fig. 1), and the patient was returned to active duty with 38 days' total sick time.

CASE 2 (Serum-Treated, Illustrative Case).—J. M. was admitted on 2/15/43 with identical and characteristic complaints. Admission temperature was 102.2° F., pulse 88, respiration 26. The examination was essentially negative. X-ray revealed atypical consolidation in the lower right lobe. Two-hundred and fifty c.c. "virus pneumonia serum" were injected intravenously on the morning following admission.

The temperature fell by crisis to normal in less than 24 hours, the findings cleared on the twelfth day, and the patient was returned to duty with a total sick time of twenty days.

CASE 3 (Serum-Treated, Illustrative Case).—W. M. C. was admitted on 3/18/43, complaining of cough and nasal congestion for past few days. Admission temperature was 100.8° F., pulse 96, respiration 20. Physical examination revealed an injected pharynx and occasional fine crepitant râles in the left chest, posteriorly. Leucocyte count was 6,200. X-ray on the day of admission disclosed an atypical consolidation of the lower left lobe.

On 3/20/43 the temperature was 101.8° F. in the morning, and 250 c.c. of convalescent "virus pneumonia serum" were given intravenously. There was no reaction. The temperature fell to normal within 36 hours (Fig. 2) and remained there, except for one reading of 99.2° F. on the eighth day. This patient was discharged from the hospital on the sixteenth day, and returned to full duty on the twenty-third day. The x-ray findings were normal before discharge from the hospital.

CASE 4 (Serum-Treated, Two Doses Required).—E. C. was admitted on 3/16/43 with complaints of chilliness and feverishness for two days; coryza and cough for several days previous; admission temperature was 102.8° F., pulse 100, and respiration 20. Physical examination revealed only a mild injection of the pharynx and occasional crepitant râles

in the right chest posteriorly. Chest x-ray was normal. Leucocyte count was 7,650. X-ray on 3/19/43, a repeat roentgenogram, revealed an atypical consolidation of the right middle lobe. At this time the temperature was 103° F.

Two hundred and fifty cubic centimeters of convalescent "virus pneumonia serum" were given intravenously. Two hours later the patient experienced a chill lasting thirty minutes and the temperature gradually rose to 104.2° F. No such reaction had been observed previously, or has been seen since, and we believe it was due to faulty preparation of the tubing.

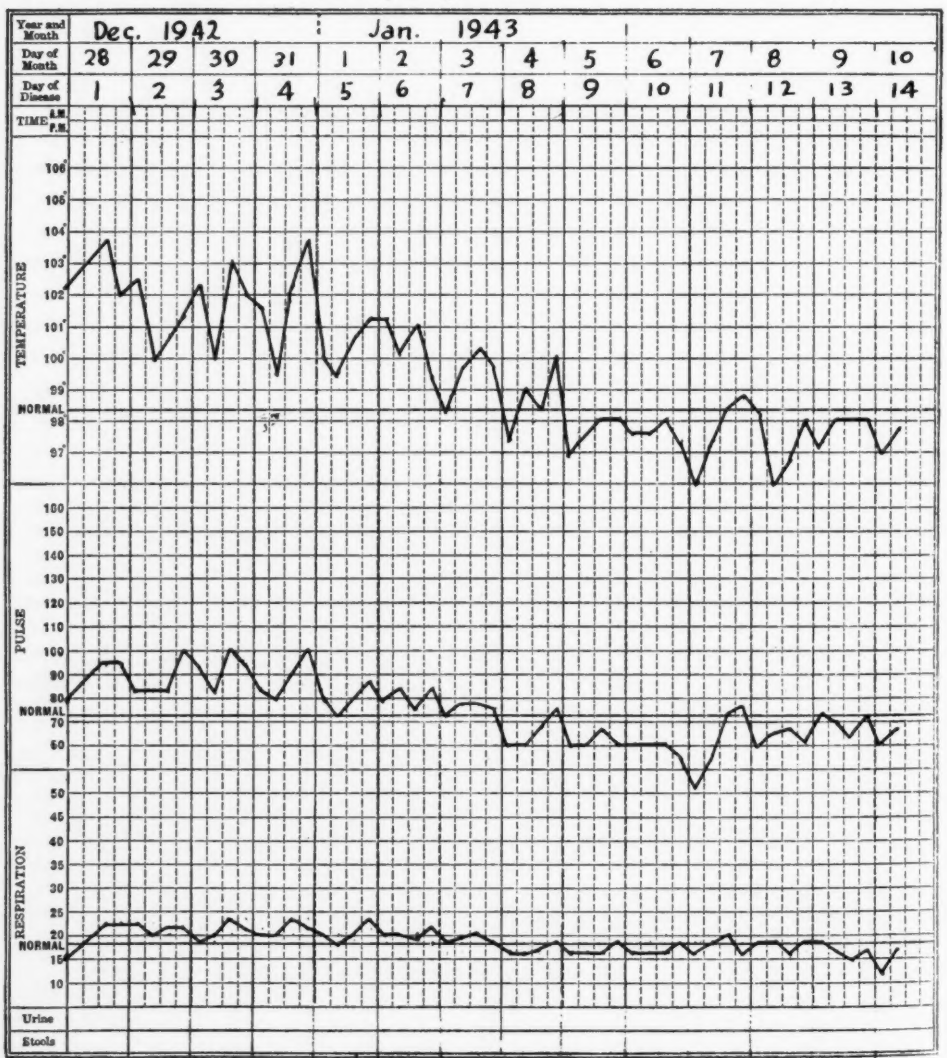
NAVAL AIR STATION
GLENVIEW, ILL.
CLINICAL CHART

N. M. S.—Form Q (1939)

Page No. .....

Name J. D. H. Rate A. C.

Diagnosis Pneumonia, Primary Atypical Race Wh. Age 22



Day after Operation may be entered in red in the space for Day of Disease.
Draw heavy lines to separate calendar days.

10—10123

Fig. 1

The temperature fell slowly by lysis but approached 101° F. three times in 72 hours. Therefore, on 3/22/43, a second unit of serum was given. There was a prompt return to normal temperature (Fig. 3), and this patient was sent to active duty on 4/8/43, a total of only 23 sick days.

Discussion and Summary.—Although this disease has a low mortality, the long hospital stay makes this no ordinary military liability. The disease inci-

NAVAL AIR STATION
GLENVIEW, ILL.
CLINICAL CHART

N. M. S.—Form Q (1939)

Page No. .....

Name W. M. C. Rate A.C.M.M.

Diagnosis Pneumonia, Primary Atypical Race Wh. Age 24

Day after Operation may be entered in red in the space for Day of Disease.
Draw heavy lines to separate calendar days.

10—10128

Fig. 2

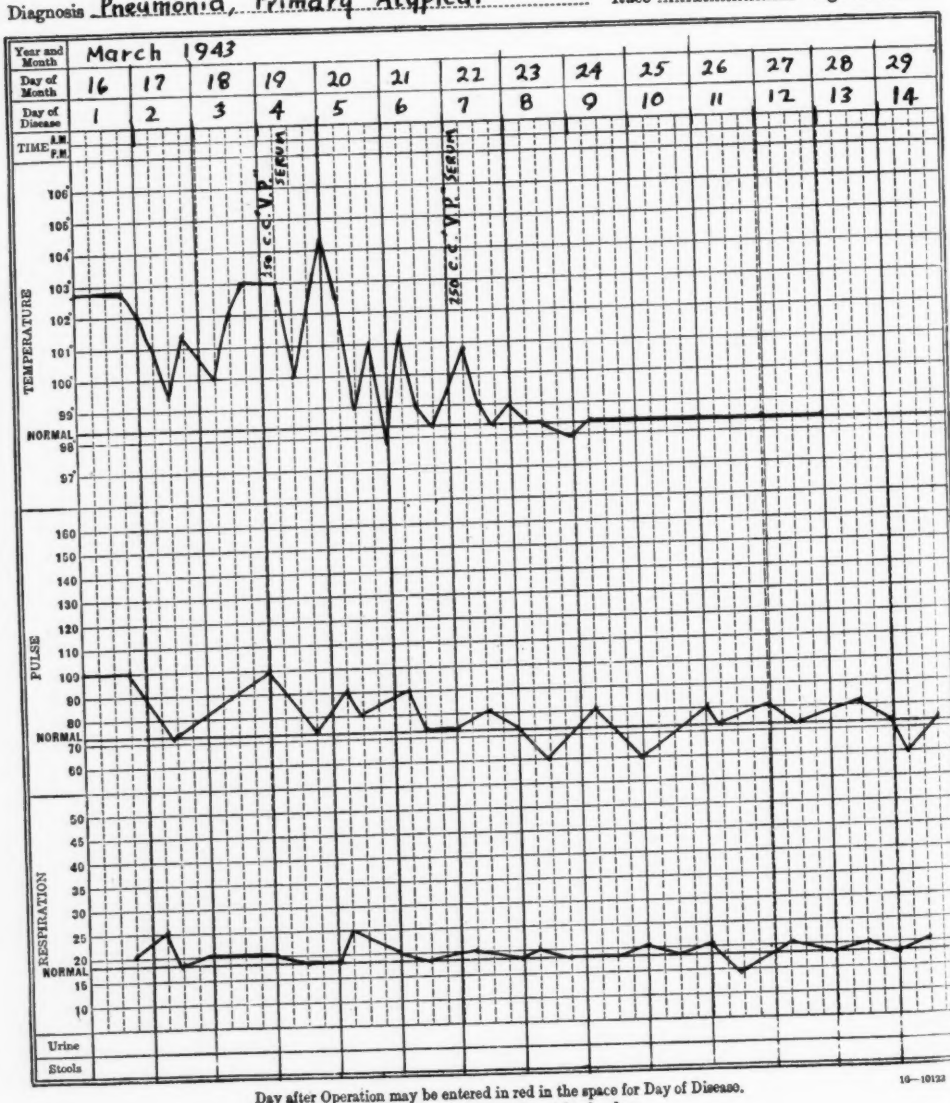
NAVAL AIR STATION
GLENVIEW, ILL.
CLINICAL CHART

N. M. S.—Form Q (1939)

Page No. .....

Name E. C. Rate S 1/c

Diagnosis Pneumonia, Primary Atypical Race Wh. Age 29



Day after Operation may be entered in red in the space for Day of Disease.
Draw heavy lines to separate calendar days.

Fig. 3

dence and severity have increased in the past several years. There is evidence to support the belief that the coming winter, particularly with large concentrations of people and mass shifting of population, may bring an epidemic of atypical pneumonia, larger in scope and more severe in nature than has been seen previously. In the absence of specific therapy, the treatment with convalescent serum yielded interesting results. Cases of atypical pneumonia which did not receive serum displayed fever lasting from five to eleven days, which dropped to normal by lysis. Convalescence was prolonged; lasting on an average of 39 days. Nine out of ten patients treated with convalescent "virus pneumonia serum" had a temperature drop to normal by crisis, and a reduction in the convalescent period to an average of twenty-two days. These patients responded to one dose of 250 c.c. of serum. Only one patient did not respond in this way, but showed a beneficial effect following a second dose of serum. Despite the rapid temperature drop to normal, and a very marked decrease of convalescent time, the days for resolution as shown by x-ray, were not shortened in proportion, although some reduction was noted.

The cases treated occurred in late winter and spring and were from our second "wave" of cases. Cases for treatment were neither chosen in succession nor in alternation with untreated cases. Rather we treated only those cases who had the most clear-cut syndrome and who seemed to be the most severely ill from a clinical standpoint. The last case treated with convalescent serum occurred in late March. We continued to see patients with this syndrome all through the month of April, which was unusually cold and wet this year in this locality.

In these late cases, due to the lack of convalescent "virus pneumonia serum," pooled adult serum in the same dosage was employed. The response in these few patients seems to be comparable to that obtained with the convalescent serum. This may be due to the fact that a large percentage of the normal adult population was antiviral substances against this, as yet unisolated, causative agent. This is the experience with certain other virus diseases. Further study along this line seems indicated, particularly in view of the fact that pooled adult serum or plasma can be obtained with relative ease.

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A CASE OF MENINGITIS IN A PREMATURE INFANT DUE TO A PROTEOLYTIC GRAM-NEGATIVE BACILLUS*

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THE occurrence of meningitis is less common in the newborn infant than in older children, and the incidence of the disease in premature infants is rare. Koplik[1] observed meningitis in twelve infants less than three months of age, and only one case in a prematurely born infant was reported. This was an infant of seven months' gestation that died of Streptococcus meningitis at the age of six weeks. Fothergill and Sweet[2] in a review of 705 cases of meningitis in children up to twelve years of age presented only three cases in premature infants. One was caused by Pneumococcus in an infant of eight months' gestation; one occurred in an infant delivered by cesarean section in the eighth month of gestation and was caused by an organism of the Alcaligenes group; the third case reported by them occurred in a premature infant of seven and one-half months' gestation and was due to *B. acidi lactici*. All these infants died. Craig[3] reported 21 cases of neonatal meningitis. Seven of these occurred in infants born four weeks prematurely, and 8 in infants born six or more weeks prematurely. None of these survived. Kutscher[4] reported a case of *Alcaligenes bookeri* meningitis, with recovery, in a four-month-old infant that had been born two months prematurely. No chemotherapy was used. In a review of 42 cases of meningitis in the newborn infant, Barron[5] did not report any cases in prematurely born infants. Holt[6] reviewed a series of 300 of his own cases of meningitis in infants up to three years of age. Only 11 were in infants under three months of age, none of which were premature. Borovsky[7] reported in 1930 that out of 190 patients with Meningococcus meningitis admitted to the Cook County Hospital in Chicago within a period of eighteen months the youngest was three months old. Cooke and Bell[8] in a report on the incidence of meningitis in early infancy reported only 10 cases under three months. None were in prematures. In a series of 136 cases of meningitis reported by McLean and Caffey[9] the youngest case included was a twenty-three-day-old full term infant. In 1926 Neal[10] reviewing the literature on meningitis caused by the bacilli of the colon group presented 118 cases and none were in prematurely born infants. In 1935 Ravid[11] reviewed 121 cases of meningitis seen in the New York Post-Graduate Hospital in the four-year period from 1928 to 1931, and only eight were in infants under three months. None occurred in prematures. Cooperstock[12] reported 14 cases of meningitis under one year of age and none were in premature infants.

We are reporting a case of meningitis due to a proteolytic Gram-negative bacillus in a prematurely born infant treated successfully with sulfathiazole. This case is of particular interest because of the unusual organism involved, the use of chemotherapy, and the subsequent recovery.

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CASE REPORT

J. P., a nine-day-old premature female infant, was admitted to the Extramural Premature Nursery of the Long Island College of Medicine Division at Kings County Hospital on Dec. 19, 1942, because of jaundice, discharging eyes, and weight loss.

The family history was noncontributory. The mother was 24 years old, and there had been no previous pregnancies. The antenatal history was interesting. When the gestation had reached four months, the mother who was unaware of the pregnancy underwent an operation for a uterine tumor. The pregnancy was discovered and allowed to continue. The pregnancy progressed uneventfully until the eighth month when the mother went into spontaneous labor and delivered a 5 pound, 5 ounce, female infant by vertex presentation at another hospital. At the time of delivery the mother was in excellent health.

After birth the infant fed poorly despite changes in the formula. During the week prior to admission the weight fell to 4 pounds and 13 ounces. On the fifth day the child became jaundiced and appeared weak. On the day before admission the infant developed a purulent nasal discharge and edema of the periorbital region. A diagnosis of acute ethmoiditis and sepsis was made and the child transferred to Kings County Hospital.

On admission the child was nine days old. She appeared jaundiced, weak, and had a feeble cry. The admission temperature was 95° F. and the weight was 4 pounds and 12 ounces. There was a moderate amount of nasal discharge, but no periorbital swelling was noted. There was a slight discharge from both eyes. The buttocks were red and inflamed. The umbilical stump was moist.

The infant was admitted to the Extramural Premature Nursery and placed in an incubator. A urine analysis on the second hospital day was normal. On the same day a blood count showed 13 Gm. of hemoglobin and 14,000 white blood cells per cubic millimeter with 46 per cent polymorphonuclear cells and 54 per cent lymphocytic cells. The icterus index was 66 units. Cultures of the discharge from the eyes revealed diphtheroids. Culture of the drainage from the umbilical stump was positive for *B. proteus vulgaris, Staphylococcus albus,* and a few colonies of nonhemolytic Streptococcus.

For the first week the patient did poorly. All feedings were taken with difficulty and the weight continued to fall despite gavaging. On the ninth day in the hospital it was observed for the first time that the child held the head retracted and had a high pitched cry. At the same time the temperature rose to 102° F. and the infant appeared much weaker. A lumbar puncture was performed with difficulty and blood-tinged fluid was withdrawn. The presence of red blood cells in the cerebrospinal fluid was attributed to trauma in performing the tap. A specimen of the fluid was sent to the laboratory for culture and reported positive for Gram-negative bacilli.

On the eleventh day the spinal tap was repeated and cloudy xanthochromic fluid was obtained. The fluid contained 500 white cells per cubic millimeter with 95 per cent polymorphonuclear leucocytes. The sugar content was too low to estimate quantitatively. A culture of the fluid was again reported positive. A blood culture taken on the same day was also positive for the same organism. On the twelfth day the child developed twitchings of the arms which lasted only a short time and did not recur. On the sixteenth hospital day the spinal fluid contained only 30 cells per cubic millimeter with 50 per cent lymphocytes. The sugar was 17 milligrams per 100 c.c. A culture of the fluid was still positive. On the twenty-second day the fluid was clear and the cell count was 215 per cubic millimeter with 100 per cent lymphocytes. The sugar had risen to 25 milligrams per 100 c.c. A culture was sterile for the first time. A blood culture was iikewise sterile. A final spinal tap on the thirty-first day was again sterile. The cell count was 65 per cubic millimeter. The sugar was 58 milligrams per 100 c.c.

Several attempts to isolate the same organism from the stools were unsuccessful.

On the tenth day the patient was started on sulfathiazole by mouth. She received 0.25 Gm., daily until the thirteenth day when the dose was increased to 0.50 Gm. daily. This was continued until the thirty-second hospital day. During this course of chemotherapy the blood titers ranged from 1.3 to 6.3 milligrams per 100 c.c.

On the third, thirteenth, and thirty-first days the infant received transfusions of whole citrated blood.

For the first three weeks the child was weak and pale. The temperature ranged from 99° F. to 102° F. At the end of this time it returned to normal and with the exception of an isolated rise on the thirty-first day following a transfusion it remained normal. The admission weight of 4 pounds and 12 ounces fell to 4 pounds and eight ounces. On the sixth day breast milk feedings were started and on the ninth day the weight began to rise. The gain was progressive. The icterus gradually subsided.

On the fortieth hospital day, at the age of forty-nine days, the infant was discharged in excellent condition weighing six pounds and four ounces.

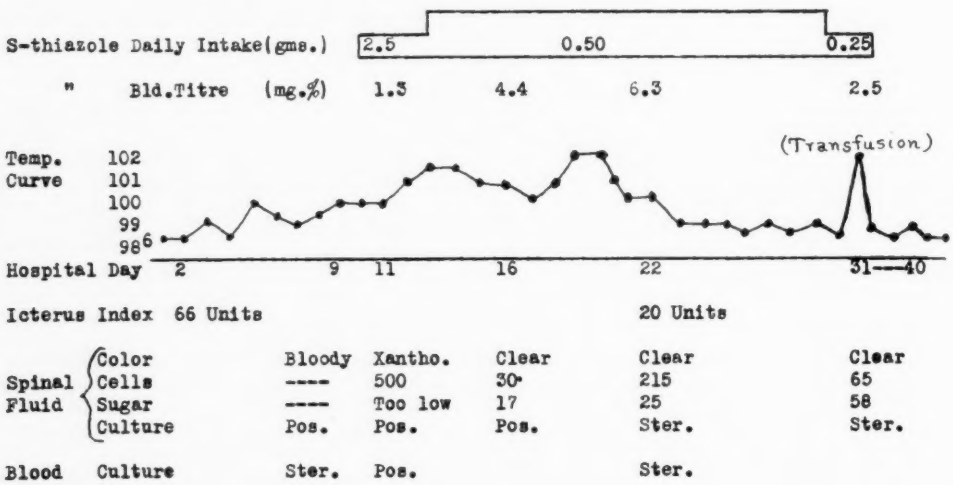

Chart I.

BACTERIOLOGY

The microorganism recovered upon one occasion from the blood and from three consecutive cerebrospinal fluids of the patient, J. P., was a nonmotile, short, straight Gram-negative bacillus with rounded ends. The bacilli occurred singly and were uniform in appearance in young cultures, but long filamentous forms were found with regularity in old fluid cultures. No branching or involution forms were observed. The organism was not acid-fast and stained readily and evenly with all the usual dyes. Flagella, endospores, capsules, and metachromatic granules were absent.

Growth was abundant on all ordinary media and was not enhanced by the addition of accessory substances. Surface colonies on agar were discrete, circular, low-convex, and amorphous with smooth surface and entire edge. There was no hemolysis or discoloration on rabbit blood agar. After 24 hours' incubation at 35° C. the diameter was 2.5 to 3.0 mm. and the color was gray with a yellowish tint. On gelatin, colonies were larger in size, irregular in shape, and medium granular in structure. Liquefaction was rapid and in stab culture was infundibuliform, extending to the base of the inoculation. Löffler's blood serum was partly digested in five days.

Chromogenesis on extract agar and on glycerol peptone agar was limited to the slight yellow of young cultures which deepened to lemon-yellow after long incubation. Neither fluorescence nor diffusing pigment was seen at any

time. Growth on potato was abundant and the yellowish-gray color present early became brown with browning of the medium in two weeks. These yellow and brown pigments were not soluble in water, alcohol, chloroform, or weak alkali.

The organism was aerobic, facultative anaerobic with an optimal growth temperature of 35° C. though it grew easily at 22° C. It was killed by exposure in a water bath to a temperature of 56° C. for 30 minutes. Growth was not inhibited by crystal violet, brilliant green, or sodium tellurite, but was prevented by bile salts in the concentrations commonly used in differential media.

Litmus milk showed an alkaline reaction; litmus was reduced except for a blue surface ring; a soft coagulum with serum layer was formed and subsequently peptonized. Indole, acetyl-methylcarbinol, and hydrogen sulfide were not produced. Nitrates were not reduced. Methyl-red and citrate agar tests were negative. Starch was hydrolyzed and tests for catalase and methylene-blue reductase were strongly positive.

No visible acid or gas was produced over a period of four weeks in peptone-carbohydrate broths; however, when the substrates were incorporated in a synthetic medium (0.2 Gm. magnesium sulfate, 0.1 Gm. calcium chloride, 0.2 Gm. sodium chloride, and 0.2 Gm. dipotassium phosphate per liter of distilled water)[17] with brom cresol purple indicator definite reactions as shown by distinct color changes were obtained. Glucose, mannitol, lactose, maltose, glycerol, salicin, levulose, trehalose, mannose, and dextrin were fermented with the formation of acid only in seven to ten days, while xylose, sucrose, arabinose, raffinose, cellobiose, rhamnose, adonitol, galactose, inositol, inulin, dulcitol, and sorbitol were not affected. It is interesting to note that while the methyl-red test was negative, the final pH of glucose-synthetic medium was 4.2.

Macroscopic agglutinations were negative with all available diagnostic immune serums in dilutions 1:20 through 1:320. Antiseruma of the Shigella, Eberthella, Salmonella, and Brucella groups, and *Pasteurella tularensis* were employed.

In a small series of laboratory animals, pathogenicity was found to be of a low degree. Guinea pigs survived an intra-abdominal inoculation of 1 c.c. of an 18-hour broth culture. Of 6 mice inoculated intra-abdominally with 0.5 c.c. of the culture, one appeared sick on the eleventh day and succumbed on the twelfth. The test organism was recovered from the heart's blood, pericardial exudate, spleen, and from small abscesses in the liver and mesentery. A second mouse became sick on the twenty-seventh day and was sacrificed. Cultures of the heart's blood were negative, but the enlarged spleen and an abscess in the liver yielded pure cultures of the bacillus. The remaining 4 mice survived. The organism was not altered in any way by mouse passage.

Among the organisms causing meningitis many varieties of Gram-negative bacilli have been reported. Of these, with the exception of *Hemophilus influenzae*, bacilli of the colon group were noted most frequently especially in infants. Barron[5] found that in 42 cases in infants under 5 months, 14 were caused by *Escherichia coli* and one each by Friedländer's bacillus, typhosus, lactis aerogenes, pyocyaneus, and *H. influenzae*. Neal[10] reported 50 cases under

3 months of which 5 were due to Gram-negative bacilli, 3 to *E. coli*, and 2 to *H. influenzae*. The remainder were tuberculous or coccal. Of 21 neonatal meningitides reported by Craig,[3] 10 were due to *E. coli* and 11 were coccal. Rauch and Krinsky[13] in 1940 concluded that although coli meningitis is rare when all meningitides in infancy and childhood are included, "coliform bacilli" are the commonest cause in infants under three months. Case reports have cited as other etiologic agents of meningeal infection, bacilli of the Salmonella, Proteus, Brucella, Pasteurella, and Alcaligenes genera and Koch-Weeks bacillus of the Hemophilus group. In others the causative bacillus was not identified.

The difficulties to be encountered in identifying a culture without accepted suitable criteria are obvious. Antigenic analysis is not always possible, and while an organism may seem to belong in a given classification with morphology as a criterion, it may be excluded from that same classification on the basis of physiologic characteristics. In the case herewith reported, the authors believe that any classification of the organism which for convenience is referred to as the "J.P." bacillus would be arbitrary and possibly erroneous, and they prefer to present the bacteriology in detail with comment upon similar described species. As tests for carbohydrate fermentation by these organisms were carried out in peptone-containing preparations used in routine investigations by most workers, it is necessary to use the negative reactions obtained with the "J.P." bacillus in this type of medium as a basis for comparison and to regard it as a "nonfermenter."

In 1933 Fothergill and Sweet[2] included in 9 cases of "colon group" meningitis one in which the bacillus recovered fermented no sugars and produced a slight yellowish pigment. Other data were not given.

Hazen and Mortillaro[14] in 1936 reported a fatal septicemia in an adult female caused by a "Hitherto Undescribed Microorganism of the Alcaligenes group." Their bacillus, although nonmotile, M.R.-negative, V.P.-negative, indole-negative and nonfermenting, was not proteolytic, did not change the reaction of litmus milk, and had variable pathogenicity for guinea pigs but none for mice.

A motile proteolytic member of the genus Alcaligenes, *A. bookeri*, an intestinal inhabitant, was recovered from a case of meningitis by Kutscher[4] in 1937. *A. bookeri* differs from the "J.P." bacillus in being motile and reducing nitrates to nitrites. There is a tendency in many laboratories to classify readily cultivable nonpigmented and nonfermenting Gram-negative bacilli, especially those of fecal origin, as Alcaligenes. Although this seems a possible genus in which to place the "J.P." bacillus, we do not feel justified in doing so. The genotype, *A. fecalis*, has always been defined as peritrichous and rod-producing, neither acid nor gas in any of the usual test substances. The possibility that *A. fecalis* might utilize glucose in such a manner that the reaction could not be detected by the usual culture methods was investigated by Conn.[15] From tests on synthetic media of nonfermenting soil bacilli and 23 strains of nonfermenting intestinal bacilli from 6 different sources, he concluded that, "The weight of evidence from this comparison of cultures is that *A. fecalis*, as generally recognized, does not grow on the particular synthetic medium

selected, and unlike the soil forms, cannot make use of glucose on such a medium. There is no evidence at hand to indicate that *A. fecalis*, as represented by the bulk of cultures received, can grow without organic nitrogen."

The "J.P." bacillus is analogous to the soil forms and inconsistent with the intestinal forms in its ability to utilize glucose and inorganic nitrogen as indicated by abundant growth on this medium.

One of the nonintestinal Alcaligenes, *A. marshallii*, described in Bergey's Manual[16] as a proteolytic nonmotile milk bacterium with yellow chromogenesis, parallels closely the "J.P." bacillus in the morphologic and physiologic properties recorded, but the description is meager and the species is not well defined. Conn[15] in a treatment of the "Validity of Genus Alcaligenes" has recommended that this species, along with other nonintestinal Alcaligenes, be transferred to "an appendix of indefinitely described species."

Of bacilli reported in cases of meningitis and described as forming no visible organic acid from carbohydrates, the "J.P." bacillus resembles most closely the one reported by Cooke and Bell[8] in 1922 as a "distinctly new type of organism" and for which they proposed the name "*Bacterium elasticum.*" With the exception of *B. elasticum's* viscid type of growth and its greater virulence for mice, the two bacilli possess like properties. By intra-abdominal injection *B. elasticum* was fatal to mice in twelve hours, and a small capsule was demonstrated in smears of the mucoid peritoneal exudate. The organism was nonmotile, strongly proteolytic, peptonized milk with little change in reaction and had no virulence for guinea pigs. There was no fermentation with gas of any sugars, but small amounts of acid with some were detected by changes in pH from 7.5 to 7.0-6.6, but never lower. These observations were made from peptone-containing cultures. In this type of medium we also noted that with some sugars pH reactions were lowered slightly.

Elrod and Braun[17] with synthetic preparations found that *Pseudomonas aeruginosa* attacked glucose, xylose, and arabinose, although Topley and Wilson,[18] Sandiford,[19] and Zinsser and Bayne-Jones[20] stated that glucose only was fermented. These studies suggest the possibility that some of the so-called "inert" proteolytic alkalinizing bacteria might prove to be acid-producing if methods other than those in general use were applied.

Similarities of a physiologic nature may indicate a relationship between the "J.P." bacillus and the Pseudomonadeae consisting of the pyocyanigenic and fluorescigenic bacilli and the plant pathogens, Phytomonas. Many of the phytomonas form green fluorescent pigment, but others may be yellow or white. It is significant to us that Elrod and Braun[17] found 2 strains of *Pseudomonas polycolor* to be indistinguishable from strains of *Ps. aeruginosa* and that many authorities agree that differentiation of *Ps. pyocyanea* and *Ps. fluorescens* is often impossible. The pathogenic potentialities of *Ps. aeruginosa* are well known, and it has been reported frequently in cases of meningitis. Although Bergey et al.[16] state that this organism produces indole, all of the fifty strains tested by Sandiford[19] were negative; indole was not formed by any of the pathogenic strains studied by Zinsser and Bayne-Jones[20] nor by the 15 strains of Elrod and Braun.[17] The latter authors state that rapid liquefaction of gelatin and failure to form indole are considered characteristic reactions of the species,

and authorities do agree in describing *Ps. aeruginosa* as motile and capable of liquefying gelatin.

Dissociants may arise, however, which show loss or alteration of one or more essential characteristics. Sonnenschein (Zinsser and Bayne-Jones[20]) described a mucoid encapsulated variety in 1927. Blanc (quoted by Hadley[21]) produced by the "modifying" action of filtrates a strain in which both pyocyanine and fluorescein were suppressed with a concurrent loss of the power to liquefy gelatin. Gessard (Hadley[21]), by the use of heat or animal passage, obtained from a strain with both pigments one producing fluorescein only. Hadley[21] described variform types derived from a single lysogenic strain. His resistant nonlytic "R" type, capable of reproducing its own type only, was always nonpyocyanigenic and sometimes achromogenic. Loss of pigment was accompanied by depression of proteolytic activity. This correlation was not found in the "J.P." bacillus in which feeble pigmentation was associated with a strongly active proteolysis.

It is well recognized that under artificial conditions bacterial transmutation may give rise to types which scarcely resemble the "parent" strain. In medical bacteriology the primary isolation of atypical strains is not uncommon and presents only temporary taxonomic difficulty if the dissociation is of the reversible type. If, on the other hand, the variant were of the relatively permanent type retaining its aberrant properties under diverse conditions, classification of the variant and identification of the "original" species might prove impossible. Over a period of five months there has been no change in the characteristic morphology and reactions of the "J.P." bacillus. Although it is our impression that a close relationship to those allied families, the Rhizobiaceae and the Pseudomonadaceae, is probable, a correct identification cannot be made at this time.

SUMMARY

A case of meningitis due to a proteolytic Gram-negative bacillus in a seven-month premature infant is reported.

The successful use of sulfathiazole is described.

A detailed bacteriologic investigation of the organism with a discussion of similar Gram-negative bacilli is presented.

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STERNAL BONE MARROW IN THE AGED*

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DURING the past few years a great deal of attention has been focused upon the medical problem of the aged. This is due in no small part to the fact that improved methods of preventive and therapeutic medicine have increased life expectancy by sixteen years.

The most pronounced pathologic changes seen in the aged are those of atrophy. The internal organs decrease in size, the liver becomes a flabby shrunken organ, and the bones become rarified.

The formative tissue of the blood, the bone marrow, is the most active tissue of the body, from infancy to old age. The red and white cells are being continually destroyed and replaced, and the bone marrow must always be ready to regenerate at great speed, to meet any unforseen demand for granular or erythroid cells.

It would, therefore, seem of importance to study the bone marrow in the aged under varying conditions, especially in regard to possible atrophic change.

To accomplish this, one hundred old patients, both male and female, sixty-five to ninety years old, were selected for study from the clinic wards of a large city hospital.

Sternal punctures were done in the usual way. A small spot of iodine was painted on the midsternal area opposite the second interspace. Novocaine was injected, and a 16 gauge needle was tapped into the marrow cavity. Four c.c. of marrow were aspirated and placed into a tube prepared previously by evaporating one c.c. of 1.4 per cent sodium oxalate to dryness.

Total nucleated cell counts were done. The material was then centrifuged, the plasma was discarded, and cellular material recentrifuged in a thin tube. The marrow layer was then removed and smears were made and stained thirty minutes by Wright's stain.

Differential counts of the smear were not done owing to the inaccuracy of the method. Instead, the smear was examined just as a section of tissue is examined and judged in regard to megakaryocytes, granular cell, and erythroid activity.

At the same time, hemoglobin and white cell determinations were done on the same patients to check on the concomitant changes in peripheral blood.

A study of the experimental data reveals that the bone marrow remains relatively normal in the aged. This is rather surprising at first since the general tendency in old age is one of atrophy. On second thought, however, it is

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easy to see that life could not continue very long after the bone marrow became atrophic, and therefore survival to old age requires a good functioning bone marrow. In some of the very old patients the cellular activity of the marrow was not only normal, but definitely increased.

It is also of interest to note that most of the old patients had an increased white count. In some cases this was due to some concomitant pathologic process, but the response was usually one of increase rather than decrease of white cells.

This observation coincides with our findings in animals. In this work we found that rats which lived longest had normal higher white counts than those with shorter life span.

It is possible that the same pertains to human beings and that one of the reasons that some persons live longer than others is that they are endowed with a better bone marrow.

CLINICAL CHEMISTRY

ALLOXAN DIABETES IN DOGS*

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SHORTLY after Bailey and Bailey[1] reported their production of diabetes in rabbits by alloxan, I undertook a number of experiments intended to elucidate whether the same was also possible in dogs. When the results that are now being published had already been found, Dr. C. C. Bailey, in a private letter, kindly made me aware of the fact that alloxan diabetes in dogs had already been reported by Brunschwig et al.[2] This knowledge gave me the impression that since alloxan diabetes in dogs is already a published fact, my results were devoid of any special interest and did not deserve publication until they were followed by many others, as a result of further experimental work on the same subject. However, a recent reconsideration of the facts that I had found a few months ago makes me feel now that, aside from the scientifically irrelevant detail of priority in the publication of the mere fact of alloxan diabetes in the dog, there is nothing, either in Brunschwig, Allen, Goldner, and Gomori's first report,[2] or in the more recent paper by Brunschwig, Allen, Owens and Thornton,[3] to deprive my results of whatever interest they might have in themselves, and since I think that, in fact, they may be found of some interest, I made up my mind to submit them for publication.

METHODS

Alloxan was injected, as will be specified, into five dogs. Except in one of them that died in less than half an hour, blood sugar was determined before the injection, and two or more times afterwards, within the next twenty-four hours. The animals were continuously watched for five to nine hours after the injection. Three of the dogs lived for several days, and one of them is still alive, five months after the injection. Unless otherwise stated, the dogs were fed, once a day, an unselected, mixed, and copious diet (restaurant waste); several fasting blood sugar determinations were made on different days; urine was collected, sometimes in 24-hour periods, and, at other times, two portions were separated in the 24 hours, one a few hours after feeding and another one that might be taken as fasting urine. Several urine examinations were made for dextrose and ketonic bodies. From two of the dogs, pancreatic biopsies were taken, when alloxan diabetes had been produced in them. From the other three, pancreatic specimens were taken post mortem. The histologic studies that have been performed by Dr. V. Suárez-Soto of this university will be published elsewhere and

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will be given only the slightest consideration here. Dextrose tolerance tests were performed on two of the diabetic animals; for comparison, a similar test was performed on one of the dogs of this series before the alloxan injection was administered, and also a dextrose tolerance test was performed on a sixth intact dog, as a normal control.

Blood sugar determinations were made by a slight personal modification[4] of the well-known Hagedorn-Jensen method. Urinary sugar was tested with the Benedict qualitative reactive; and ketone bodies by the Legal reaction; results for both, when positive, are given by means of plus signs from one to four. To avoid repetition, it will be stated only here in the article, that blood sugar figures are given in milligrams of dextrose per 100 c.c. of whole venous blood. In Tables III, IV, and VI, "fasting blood sugar" means, not only fasting, but before any injection is given. In Tables I and II, "before" means just before (within three minutes) the beginning of the injection of alloxan or the beginning of the milk with dextrose drinking by the animal, respectively. In Tables I and VII, the time figures, except when otherwise stated, mean moments at the end of the stated time lapses, after the injection of alloxan, counted from the end of the last injection. In Table II, the time figures mean moments of blood sampling, at the end of the stated time lapses, counted from the moment the animal finished drinking the milk with dextrose.

RESULTS

For the sake of saving space, together with clarity, the results are given only in tabulated form.

TABLE I

EARLY EFFECTS OF ALLOXAN UPON BLOOD SUGAR. THE INJECTIONS TO DOG 5 WERE GIVEN DURING THE FOLLOWING INTERVALS: FOUR DAYS BETWEEN FIRST AND SECOND SUBCUTANEOUS; THREE DAYS BETWEEN THE SECOND SUBCUTANEOUS AND INTRAVENOUS. WHEN GIVEN THE ALLOXAN INJECTIONS, ALL DOGS, EXCEPT DOG 3 (SEE TABLE V), HAD BEEN FASTED FOR NOT LESS THAN 20 HOURS AND WERE KEPT FASTING UNTIL AFTER THE LAST BLOOD SAMPLE, ON THE SAME DAY OF THE INJECTION, WAS TAKEN, WHEN THEY WERE FED

DOG NUMBER:	1	2	3	5	5	5
Milligrams of alloxan injected per kilogram:	100	100	300	75	75	200
Kind of injection:	intravenous	intravenous	intravenous	subcutaneous	subcutaneous	intravenous
Before:	102	105	93	110	105	121
15 minutes	102					
30 minutes			90			
2 hours	140			91		
2½ hours		106				99
3½ hours	124					
4 hours			220	83		
5½ hours					105	
8 hours				81		
8½ hours	75					76
9 hours		87				
24 hours	343	125				281

COMMENT

The Production of Diabetes in Dogs by Alloxan Has Been Confirmed.—Not only glycosuria and hyperglycemia in Dogs 1, 2, and 5, but also polydipsia and polyuria in Dogs 1 and 5 and severe weight loss in Dog 1 can leave no doubt

about this point. Further evidence is afforded by the dextrose tolerance tests (Table II), that of Dog 1 corresponding to a rather severe diabetes and the first test of Dog 2 corresponding to a very mild one.

Curability of Alloxan Diabetes.—Dog 2, after exhibiting mild glycosuria and hyperglycemia for a few days, as a result of an injection of alloxan, and giving a mild diabetic dextrose tolerance test, not only had, shortly afterwards, a permanently sugar-free urine, but, 42 days after the alloxan injection, gave a perfectly normal dextrose tolerance test. This animal, therefore, as a result of

TABLE II

BLOOD DEXTROSE TOLERANCE TESTS, BOTH ON ALLOXAN-INJECTED DOGS AND ON NORMAL CONTROLS; 1.5 GM. DEXTROSE PER KILOGRAM DISSOLVED IN 15 C.C. MILK PER KILOGRAM WERE ORALLY ADMINISTERED TO THE FASTING ANIMALS. BLOOD SUGAR WAS DETERMINED BEFORE THE ANIMALS DRANK THE MILK WITH DEXTROSE AND AFTERWARDS, AT THE END OF THE SPECIFIED LAPSES OF TIME

DOG NUMBER:	1	2	2	3	6
Condition of the dog:	Injected	Injected	Injected	Normal control, to be injected, later	Normal control
Milligrams of alloxan intravenously injected, per kilogram:	100	100	100		
Time after injection of alloxan:	8 days	5 days	42 days		
Before:	492	103	88	99	90
30 minutes	550	220	135	122	149
1 hour	605	220	134	148	148
2 hours	575	110	89	148	100
3 hours	480	105	87	93	82

TABLE III

MAIN COURSE OF EVENTS OF EXPERIMENTS WITH DOG 1

DATE (1943)	WEIGHT, KG. (FASTING)	FASTING BLOOD SUGAR	GLYCOSURIA		KETOSIS	REMARKS
			FASTING	AFTER FOOD		
9/2	13.0	102	no	no	no	Intravenous injection of alloxan. 100 mg. per kilogram in three portions; 15 minute intervals.
3		343	+++	++++	no	Normal-looking; very marked polydipsia and polyuria.
4	13.7	341	+++	++++	no	Rather depressed at moments.
5						Whole-day fast.
6	12.8	215				Normal-looking.
8	13.5	350				
9		492	++++	++++	++	Very marked lipemia. Dextrose tolerance test.
10	13.2					Vomits.
14				++++	++	Quite normal-looking.
18	11.7					
20	11.2					
22						Whole-day fast.
23	10.7	358	+	+++	+++	Laparotomy, under nembutal. Pancreatic biopsy.
24			+	+	+	Extremely depressed. Drinks and urinates very little. Up to now polydipsia and polyuria had been very marked.
25						Dies.

alloxan action, developed a very mild but unmistakable diabetes, and this was subsequently cured. I think that this fact is one of great interest.

Varying Degrees of Severity of the Diabetes Produced by Alloxan.—The diabetes of Dog 2 was, as we have seen, extremely mild; that of Dog 1 was of a rather severe character; it cannot be said that it was extremely severe, since no insulin was given and no food restriction was instituted, but there is no doubt that it was severe, as made evident by the dextrose tolerance test (Table II), as well as by the severity of the symptoms (Table III), including ketosis and marked lipemia. The diabetes of Dog 5 (Table VI) was a little less severe than that of Dog 1, but much more so than that of Dog 2. It is not possible to

TABLE IV

MAIN COURSE OF EVENTS OF EXPERIMENT WITH DOG 2

DATE (1943)	WEIGHT, KG. (FASTING)	FASTING BLOOD SUGAR	GLYCOSURIA		KETOSIS	REMARKS
			FASTING	AFTER FOOD		
9/4	6.5	105	no	no	no	Alloxan injected, 100 mg. per kilogram intravenously; two portions; 5 minute interval.
5		125	no	+	no	
6	6.3	136	no	++	no	
8	6.5	120	no	no	no	
9		103	no	no	no	Dextrose tolerance test.
13			no	+	no	
14			no	no	no	From this day on, instead of former mixed unmeasured food, beef lung, 400 Gm. daily.
20	7.0		no	no	no	
10/14	7.5		no	no	no	
16	7.2	88	no	no	no	Second dextrose tolerance test

TABLE V

MAIN COURSE OF EVENTS OF EXPERIMENT WITH DOG 3. THE OLD AGE OF THE ANIMAL SHOULD BE TAKEN INTO ACCOUNT WHEN JUDGING THE DEXTROSE TOLERANCE TEST (SEE TABLE II)

TIME	BLOOD SUGAR	REMARKS
9/8/43		
11:45 A.M.:	93	Last blood sample of dextrose tolerance test. Immediately afterwards, injection of first of three portions of a total dose of 300 mg. per kilogram of alloxan intravenously.
11:58 A.M.:		Injection of second portion.
12:15 P.M.:		Injection of third portion.
12:45 P.M.:	90	
4:00 P.M.:		Very depressed. While able to walk, prefers to lie down.
4:15 P.M.:	220	Paresis of the extremities, specially the anterior. The animal is thought to be hypoglycemic.
5.00 P.M.:		Semicomatose, emesis; a little later 5 Gm. dextrose are given per os. (The result of the blood sugar determination was not known until next morning.)
6:00 P.M.:		Ten grams dextrose intravenously; the condition of the animal improves considerably.
From 6:15 P.M. on:		Drinks water several times and each time vomits; falls again into a semicomatose condition.
7:45 P.M.:		Ten grams dextrose intravenously; great improvement.
9:30 P.M.:		Still in a comparatively fair condition. Ten grams dextrose subcutaneously, to prevent chances of further development of hypoglycemia.
Some time during the night:		Dies.

TABLE VI

MAIN COURSE OF EVENTS OF EXPERIMENT WITH DOG 5

DATE (1943)	WEIGHT, KG. (FASTING)	FASTING BLOOD SUGAR	GLYCOSURIA	KETOSIS	REMARKS
9/11	14.8	110	no	no	Subcutaneous injection of alloxan; 75 mg. per kilogram.
13			+	no	
14			no	no	
15			no	no	Second subcutaneous injection of alloxan; 75 mg. per kilogram.
16, 17			no	no	
18		121	no	no	A subcutaneous induration in the site of the last injection is noticed. No tenderness. No signs of pain or discomfort. Intravenous injection of alloxan; 200 mg. per kilogram, in two portions; 20 minute interval.
19		308	+++	no	Polydipsia and polyuria.
20	14.5	281	+++	+	The induration has softened; seems to be an abscess and to contain gas. Still no tenderness.
21					Animal seems to be very ill; eats very little.
22			+++	+	Liquid stools. Abscess breaks. Animal dies.

TABLE VII

EARLY REACTIONS OTHER THAN BLOOD SUGAR CHANGES ELICITED IN SOME DOGS BY THE INTRAVENOUS INJECTION OF ALLOXAN

DOG NUMBER:	1	3	4
Milligrams of alloxan intravenously injected per kilogram:	100	300	300
Number of portions in which total dose was distributed:	3	3	1
Time intervals between injections:	15 minutes	15 minutes	
Time of appearance and description of reactions:	10 minutes after second and 5 minutes before third injection; emesis. From 30 minutes until 2½ hours after the last injection, the animal is slightly depressed, lying down most of this time, although being able to walk. From the end of 2½ hours after completion of the injection, the animal looks and behaves quite normal.	3¾ hours after completion of injection the animal is depressed and lying down, although able to walk. 4 hours id. id., paresis of extremities, especially anterior, and seems to be hypoglycemic, although blood sugar is 220. Five hours id. id., lying down, semicomatose. 5¼ hours id. id., emesis; condition improved by dextrose administration. 6½ hours id. id., repeated emesis after drinking water; becomes worse and again improves after dextrose. 9¼ hours id. id.; fairly good condition. Some time later, dies.	Immediately after the injection: emesis; extremely depressed. Dies 25 minutes after completion of injection.

say whether the observed degree of severity of the disease in Dogs 1 and 5 would have remained more or less unchanged had the animals remained alive. This is an extremely important point that remains to be elucidated by further research. If the severity of alloxan diabetes is, in some cases at least, of a reasonably stable degree, I think that the possibility of producing diabetes in a wide range of varying degrees of severity may prove a fact of great importance.

The Early Blood Sugar Changes Elicited by Alloxan in the Dog.—The fact that in three of five alloxan-injected dogs that lived long enough to become diabetic there was no need to give them any special protection against hypoglycemia in order to keep them alive is in sharp contrast with the death in hypoglycemia of nearly all the rabbits in Dunn and his co-workers' experiments[5] and with the strenuous fight against hypoglycemia that Bailey and Bailey had to carry on in order to keep their rabbits alive. The figures in Table I show that, although in dogs after alloxan there is, in general, a certain fall of the blood sugar level, this fall appears much later and is much less marked than in rabbits. None of the figures found in any of the dogs in this series may be called hypoglycemic. So far as the initial hyperglycemia is concerned, a typical and constant feature of the rabbit reaction to alloxan, only in Dogs 1 and 3 did I find a comparatively early rise of the blood sugar level. It is true that my blood sugar determinations were not frequent enough to make it certain that there were no other high figures. In any event there can be no doubt that, in the dog, the initial hyperglycemia, produced by alloxan, when it exists, begins later than in the rabbit and, on the other hand, lasts longer, and there is, at least, some reason to believe that the initial alloxan hyperglycemia in the dog, as a rule, is much less marked than in the rabbit.

Reactions Other Than Blood Sugar Changes.—Except for the very early death of some of Dunn and his co-workers' animals,[5] from all that has been published about rabbits it might be gathered that the reactions described by both Dunn and his co-workers[5] and Bailey and Bailey[1] are merely those associated with hypoglycemia. Brunschwig and his co-workers[2, 3] do not mention any reactions in their dogs, except early death, but in human subjects on which they have used alloxan at very high doses, reactions such as nausea, emesis, chilling, and icterus are mentioned. In three out of five dogs of the present series, reactions other than those generally associated with blood sugar changes were observed (Table VII). In Dog 1, emesis was elicited by only 66 mg. alloxan per kilogram, and after the 100 mg. per kilogram dose was completed, there was a short period of slight depression, the whole thing being in contrast with the total absence of any visible reaction in Dog 5 (Table VI) after a dose of 200 mg. per kilogram. Dog 3 showed a very severe reaction following a dose of 300 mg. per kilogram. Of special interest is the fact that, at a moment when the blood sugar was 220, I mistook the condition of the animal for typical hypoglycemia, and my erroneous conviction was reinforced by the fact that dextrose administration repeatedly improved the condition of the animal. Although no further blood sugar determinations were made and the animal died during the night, while not being watched, I am convinced that this dog's death was not due to hypoglycemia, as certainly was not that of Dog 4.

Wide Variation of Individual Reactiveness to Alloxan.—The same dose of 100 mg. per kilogram produced a severe diabetes in Dog 1 and a very mild and curable one in Dog 2. This is in agreement with facts described in rabbits by previous observers. Brunschwig, et al.[3] state that 200 mg. per kilogram are invariably fatal to dogs, but Dog 5 of the present series (Table VI) stood that dose without the slightest untoward reaction and developed a moderately severe diabetes; although it died within a few days, its death cannot be ascribed to a toxic action of alloxan.

Pancreatic Changes.—These will be extensively studied somewhere else. Suffice it to say here that, in a general way, our findings are in agreement with those of previous observers, and that in the pancreas specimen taken by biopsy from Dog 2, when it was unquestionably diabetic although in a very mild degree, no islet or other lesions were found. This shows that a noxious agent can elicit a diabetic functional disturbance of the endocrine pancreas even when no associated anatomic lesion may be detected, a point of interest in connection with problems of human diabetes, and in agreement with some observations of Brunschwig, et al.[3] on human subjects.

Theoretical Considerations.—When commenting about their discovery of the alloxan necrosis of the islands of Langerhans, Dunn, Sheehan, and McLetchie[5] made some very interesting suggestions. One of them was that the islet necrosis might be caused by unbearable strain from overstimulation; the primary action of alloxan would be to overstimulate the islets to produce insulin, and what in their experiments was the final hypoglycemia might be taken for evidence of that overstimulation. This hypothesis is hard to conciliate with the fact that, as a result of alloxan, dogs may, as 1 and 5 of this series, develop destructive lesions of their pancreatic islets and a severe diabetes without having, at any moment, shown either blood sugar figures or external signs of hypoglycemia. It seems more probable that the noxious action of alloxan upon the cells of the islands of Langerhans should be of a more direct and, so to say, brutal character.

SUMMARY

1. The production of pancreatic diabetes in dogs by alloxan is confirmed.

2. Alloxan can produce diabetes of varying degrees of severity.

3. The diabetes produced by alloxan, when of a very mild degree of severity, may be spontaneously curable.

4. The initial blood sugar changes brought about in the dog by diabetogenic doses of alloxan are slower in their appearance and smaller in magnitude than those in the rabbit; the initial hyperglycemia begins later; when detected, lasts longer; and the figures found in the present experiments have been, with one exception, much lower. The subsequent fall begins much later and, in the present experiments, has never been found to reach hypoglycemic levels. None of the three dogs that became diabetic required any antihypoglycemic treatment in order to be kept alive.

5. In all the three dogs that survived the injection of alloxan for more than one day, diabetes, with hyperglycemia and glycosuria, was established twenty-four hours after the injection.

6. Toxic reactions, other than those related to blood sugar changes, such as emesis, depression, paresis, and torpor, have been observed in some cases. Two dogs injected with 300 mg. per kilogram died in a very short period of time. In one of these, a condition that could be mistaken for hypoglycemia existed at a moment when actually there was hyperglycemia.

7. There is a wide range of variation as to the individual reactiveness of dogs both to the toxic and to the diabetogenic action of alloxan.

8. The hypothesis that the primary action of diabetogenic doses of alloxan on the islands of Langerhans might be one of overstimulation, resulting in temporal hypoglycemia from hypersecretion of insulin, is hard to conciliate with some of the presented facts.

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LABORATORY METHODS

GENERAL

LABORATORY AIDS IN THE DIAGNOSIS OF MALARIA*

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MALARIA is a disease of prime importance in the tropics and may soon attain this distinction in regions in the United States where it was rare before the war. In this paper we wish to describe the methods which have been used with success in making the laboratory diagnosis of malaria in an army hospital operating in the South and Southwest Pacific theatre of war. The first year of practicing jungle medicine was a trial and error period for those of us not previously expert in the laboratory diagnosis of tropical diseases. Now that this adjustment is over, we feel that we are having good results in finding malarial parasites in soldiers suspected of having the disease. This has been accomplished, in some instances, by the use of standard techniques in staining blood smears, but as a rule, we have had to alter textbook procedures to meet jungle conditions. We have learned the techniques used by New Zealand and Australian medical officers, and we have developed certain modifications of our own. It is hoped that the recording of these may contribute something to the new history of malaria that is now being written throughout the world.

CONSIDERATIONS IN THE LABORATORY DIAGNOSIS

The finding of parasites, pigmented leucocytes, and a high percentage of monocytes in the blood smear are the outstanding considerations in making a laboratory diagnosis of malaria. However, a positive diagnosis of malaria should not be made unless parasites are found in the blood smear. It is difficult to find parasites in soldiers who are receiving suppressive antimalarial therapy, even when they have typical symptoms of malaria. Atabrine has so altered the picture that in less than 40 per cent will parasites be found in the initial smear, and, in these, many parasites will be atypical in appearance. In troops operating in malarial districts it is essential to collect both thick and thin smears from every febrile patient. Ideally, blood films should be collected before any antimalarial therapy is started, but of course in soldiers who are receiving suppressive therapy, this is impossible. If smears are repeated at intervals from four to eight hours, we have almost invariably found parasites before the sixth smear was taken. This is usually in the thick smear. It may

*From the Laboratory—Medical Service of the Evacuation Hospital.
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take several hours of searching to find even one parasite in the thin portion of the same slide.

It is the practice of this hospital to have thick-thin blood smears taken on patients suspected of having clinical malaria upon admission to the hospital. Treatment may be started even before the smear is examined if the patient is quite ill. It is our impression that the number of parasites is not noticeably diminished during the first twenty-four hours by the low dosage atabrine therapy previously in use. However, in those who receive quinine or in those receiving massive doses of atabrine it may be impossible to find any parasites in the blood after twelve hours of therapy.

PREPARATION OF A "THICK-THIN" SMEAR

After some months of making smears too thick or too thin, we have developed a technique which works very well. A heavy mark is drawn, with a wax crayon, across a glass slide about three-fourths of an inch from one end. A finger is cleansed, dried, and pricked with a lancet. A drop of blood, about the size of a small pea, is placed in the smaller area of the slide. Utilizing the ball of the bleeding finger as a spreader, the drop is spread to the size of a five-cent piece and thin enough to see the hands of a watch through the film. A very thin film is spread at the opposite end of the slide. With the thick portion up, the slide is placed in a semivertical position to dry and the excess blood from the thick smear is allowed to drain to the crayon mark. The slide is air-dried in this position and under a screen if flies are about. In damp weather it is necessary to dry the slides in a dry hot air chamber, easily made from a tin can.

TECHNIQUE OF STAINING A "THICK-THIN" SMEAR

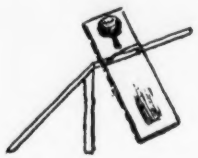
When a large number of blood examinations for malaria are performed daily it becomes necessary to develop a rapid technique for staining films. For ordinary diagnostic purposes we have adopted a rapid method of staining malarial parasites in thick smears as described by Field.[1]* The thick smear is ready for staining as soon as it appears dry (Fig. 1). The film is dipped for one second in Solution A. It is immediately dipped in clean water for a few seconds or until the stain ceases to flow from the slide. It is then dipped in Solution B for one second. The stain is flushed off and then rinsed gently in clean water. It is dried in a vertical position. There may be slight variation in timing with different batches of stain. The smear should be stained immediately after drying for the best results.

*Field's Stain, modified.

Solution A:
1. Dissolve 1.25 Gm. of methylene blue chloride and 5 Gm. of sodium phosphate dibasic, Merck, in 50 c.c. of distilled water.
2. Bring to a boil. Then evaporate to dryness. (This is the most important step in preparing a good stain.)
3. Add 6.25 Gm. of potassium phosphate monobasic, Merck, dissolved in 500 c.c. of distilled water. Mix well and let stand for a few days. Filter before use.

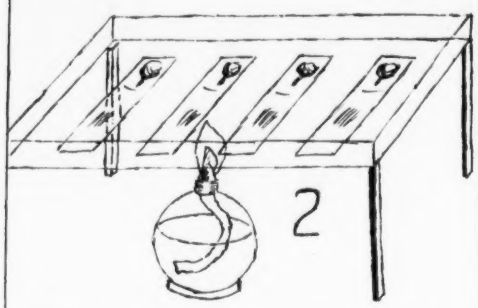
Solution B:
1. Dissolve 5 Gm. of sodium phosphate dibasic, Merck, and 6.25 Gm. of potassium phosphate, Merck, in 500 c.c. of distilled water. Add Eosin Y, 1 Gm.
2. Solution must be replaced when it becomes greenish. We have modified the original stain described by Field by omitting Azure B from mixture 1 because of difficulty in obtaining this dye. We have compared our stain with the standard Field stain as prepared for the Royal Australian medical Corps by the Commonwealth Laboratories of Australia. We are convinced that our stain compares favorably with the original in every respect.

Technique of Making a "Thick-Thin" Smear for the Examination of Malarial Parasites



1

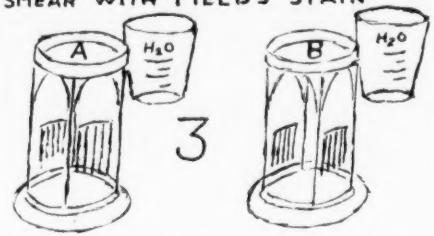
Proper method of preparing "Thick-Thin" smear



2

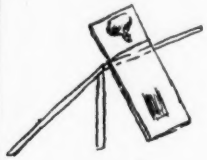
Rapid drying of slides in damp weather

Staining thick portion of smear with Field's stain



3

I. Dip thick smear in sol. A for 1 second.
II. Rinse in water.
III. Dip in solution B for 1 second, flush off.
IV. Rinse in clear water

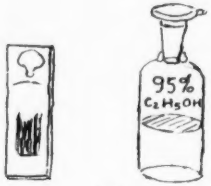


4

Air Dry

5

Stain thin portion of smear with Wright's Stain



6

Decolorize thin portion of smear until it is a light pink in color

Stain with Giemsa

7

Stain 1:15 dilution for 15-20 minutes. Slide is placed face down in the diluted stain. Wash and dry.

8

Examine thick-thin smear under oil immersion. Parasites are most numerous at the point designated above

Bent ~ '43

Fig. 1.

After the thick portion of the smear is stained and dried, the thin smear is now ready for staining. We have found that a combination of Wright's[2] and Giemsa[3] stains gives nearly perfect results for examining parasites and red blood cells. Fourteen to sixteeen drops of Wright's stain are floated in the center of the thin film. This is allowed to stand for one-half to one minute to fix the stain. Then it is diluted with twenty-four to twenty-six drops of buffer solution[4*] or distilled water. The mixture is allowed to stand for from eight to twelve minutes. The slide is washed well with water. The smear is then decolorized with 95 per cent ethyl alcohol until it is a pale pink color. Freshly filtered stock Giemsa stain is diluted with fifteen parts of the phosphate buffer or distilled water and placed in a Petri dish. The thin portion of the smear is now placed face down in contact with the stain in the Petri dish for twenty to thirty minutes. It is then washed and dried.

Frequently it is difficult to get a perfect result with Wright's stain. Because of variation in batches of dye or because the technician may overstain the smear, the red blood cells appear too blue, making it difficult to find small trophozoites. The technique described helps correct this fault.

EXAMINATION OF THE "THICK-THIN" SMEAR

The appearance of the malarial parasites in the thick smear is very different from that in a thin smear. Whereas in the thin smear, the parasite with its typical red staining chromatin dot and blue cytoplasm is found in the infected red blood cell, in the thick smear the red blood cells are destroyed leaving the parasites free and concentrated. Under oil immersion and using the 5× ocular, the thick smear is examined at the junction of the circumscribed portion and the fingerlike projection where the blood has drained toward the center of the slide. This portion is clearly seen when the slide is placed on a white background. It appears as a thin wavy brown-red line. This is not only the area of maximum dehemoglobinization, but it is at this point that the parasites tend to cling when the blood is dried with the slide in a semivertical position. The 5× ocular is used initially because it is easier to look for parasites or pigmented leucocytes in a smaller field. In a smear which contains parasites and which is stained by Field's method, the chromatin dots stain a bright red to a crimson and can be seen easily. These dots are then examined with the 10× ocular. In many instances the blue cytoplasm is found coexistent with the chromatin dots, confirming the impression that plasmodial parasites are present in the smear. In the thick smear parasites appear smaller and more shrunken than in a thin film.

Plasmodium vivax is characterized by a single red chromatin dot associated with blue cytoplasm. This has a tendency to assume many variations in form dependent on the stage of the parasite. Whereas in the young trophozoite the cytoplasm may appear as an "interrupted ring," in older trophozoites there is a decided tendency for the cytoplasm to disperse and arrange itself in clusters. In the presegmenting and segmenting schizonts the cytoplasm is more

*This buffer is prepared by dissolving sodium phosphate dibasic, Merck, 9.47 Gm., and potassium phosphate monobasic, 9.08 Gm., in distilled water, making each solution up to 1000 c.c. The proportions of acid and alkaline phosphates may be adjusted to any pH. Usually for blood smears a pH of 7.0 is desired. This is made by mixing M/15 Na_2HPO_4 61.1 c.c. and M/15 KH_2PO_4 38.9 c.c. In the tropics we have had more success using pH of 6.4. This is made by mixing M/15 Na_2HPO_4 71.3 c.c. and M/15 KH_2PO_4 28.7 c.c.

compact and the chromatin divides into several irregular masses associated with cytoplasm. The schizonts are large and have yellow-brown pigment dispersed through the cytoplasm. Frequently this pigment is found free on the background of the thick film. Merozoites number from eight to eighteen. The microgamete is large with undivided red chromatin surrounded by a small amount of light-staining cytoplasms. This contains scattered red-brown granules and yellow pigment. Macrogametes cannot be distinguished from mature trophozoites.

The *Plasmodium falciparum* consists of a single or double red chromatin dot associated with a wisp of blue cytoplasm which resembles a comma or an exclamation point. Frequently scattered throughout a smear containing falciparium organisms are single red dots without any pigment or cytoplasm. In our experience the presence of these suggests that typical parasites will be found. The gametocyte of *Plasmodium falciparum* is the typical sausage- or crescent-shaped parasite which stains light blue and has a central golden-yellow mass of pigment. It is difficult to distinguish the sex in a thick smear. The young trophozoite and the gametocyte are usually the only forms of *Plasmodium falciparum* found in the blood. However, in very severe subtertian malaria, larger trophozoites and even schizonts may be present.

Plasmodium malariae is characterized by a chromatin dot which is larger than that found in the other plasmodia, a relatively small amount of dark blue cytoplasm which tends to form bands, and a large amount of coarse brown pigment which remains compact. The gametocytes resemble vivax but are smaller and heavily pigmented. *Plasmodium malariae* has been uncommon in our studies.

Difficulties arise when infections are light, as they frequently are in soldiers taking suppressive antimalarial therapy. Under these conditions parasites may appear degenerate and stain poorly.[5] The chromatin dot may stain darkly or the cytoplasm may be a very light or unevenly stained blue. They may be easily confused with blood platelets or reticular debris. However, in our experience, one can usually find at least two typical parasites in the smear.

In the thick smear the polymorphonuclear leucocytes stain violet-blue, with fine brownish granules. If pigment is present, it is usually gathered at one end of the cell and is yellow-brown in appearance. Monocytes stain deep blue and basophiles are recognized by predominating dark blue-black granules. The granules of eosinophiles stain a flat brown-red which are darker and larger than chromatin dots. Field[6] in his original article gives an excellent description of the appearance of the malarial parasites in the thick smear, and in addition presents some photographs of typical and atypical forms of plasmodia.

Early ring forms of *Plasmodium vivax* are difficult to distinguish from the trophozoites of *Plasmodium falciparum*. This is especially true in mixed infections. The differentiation in diagnosis is assisted by the fact that the young trophozoites of *Plasmodium vivax* are nearly always associated with older forms of the parasite. *Plasmodium falciparum*, on the other hand, are more consistent in size and shape. A smear repeated in twelve hours may clarify the diagnosis by presenting definite schizonts of *Plasmodium vivax*. However, as a rule, a thin smear is necessary to determine the type of plasmodium. The thin

smear should be prepared thin enough to compare the individual red blood cells with each other and in such a manner that there is a well-defined edge parallel to and about one-eighth of an inch from the edge of the slide. Large parasitic forms, like polymorphonuclears, tend to cling to the edge. Then one can usually differentiate the fine delicate ring of the falciparum from that of the coarser vivax which is found in enlarged red blood cells.

OTHER LABORATORY AIDS

It is our experience that the total leucocyte count in malaria is between 5,000 and 10,000 cells. Before a chill the leucocyte count tends to be lowest, while during the chill or shortly thereafter there is an increase in the number of cells, particularly monocytes. Twenty per cent of the cells may contain malarial pigment. The presence of pigmented monocytes and polymorphonuclear leucocytes are almost as diagnostic of malaria as the parasites themselves.[7]

Anemia is a characteristic feature of malaria. In recurrent cases, particularly, it is not infrequent to obtain a fall of from one to two million red blood cells and a 25 per cent drop in hemoglobin. In severe cases of malignant tertian malaria, a hemolytic anemia may be encountered with a high icterus index and increased urobilinogen in the urine or feces. All patients with cerebral malaria should be blood-typed upon admission, and a blood transfusion should be given at the first indication of rapid blood destruction.

When large amounts of atabrine are present in the urine, a bright yellow color results. However, a better method to determine if atabrine is present in the urine is to concentrate the atabrine by extraction with amyl alcohol by the method described by Wats and Ghosh.[8] It has been modified to facilitate interpretation, and as a matter of economy. Ten cubic centimeters of urine are alkalinized by adding 1 c.c. of saturated potassium or sodium carbonate. One cubic centimeter of amyl alcohol is added to the solution and shaken well. It is permitted to stand until the alcohol layer separates. If atabrine is present, a yellow-green fluorescence can be seen in the alcoholic layer if examined in the sunlight. Ether may be substituted for amyl alcohol, but the yellow color is less intense. This test is of value in determining whether the patient has been taking suppressive antimalarial therapy prior to treatment, or, in those patients who have never taken atabrine prior to treatment, whether absorption and excretion is taking place. Similarly the urine can be tested for quinine.[9]

CONCLUSION

The laboratory diagnosis of malaria is not difficult. It is a question of good staining technique and examining the blood for parasites at the proper time. The rapid staining technique of thick smears as devised by Field is, in our opinion, ideal for rapid diagnosis and when a large number of smears must be examined as quickly as possible. Although many authorities feel that blood films should be dried in the horizontal position, in our opinion, when Field's stain is used, there is a definite advantage in drying the smear in the semivertical position. The parasites tend to concentrate in one area; this diminishes orientation time and facilitates examination. As far as we can determine, there are two disadvantages to Field's technique. First, if the slide is not washed

well, some eosinophilic staining debris clings to the slide. The examiner soon learns to differentiate these artifacts from parasites. Second, the stain fades, making it impossible to keep slides for permanent record. It is imperative that the thick smear be not too thick and that the smear is examined at the point where the parasites tend to concentrate. The standard or Wright-Giemsa thick smear staining techniques as described by Wilcox and Logan[10] are excellent, but are not as convenient as the technique described above. In a Giemsa-stained thick smear the chromatin dot is not as vivid, but the cytoplasm is more intense. Although it takes more time to stain a thin smear with the Wright-Giemsa combination than with a simple Wright's stain, it is well worth the effort in those instances where it is difficult to find a parasite or identify the type of plasmodia present. If the slide is stained face down in a Petri dish, the debris and sediment will fall off and a clean blood film will result.

SUMMARY

1. The laboratory routine used in the diagnosis of malaria which has been performed for over a year by a large army hospital in the South and Southwest Pacific theatre of war is described.

2. A rapid technique for staining malarial parasites in thick smears, which has received little attention in the literature, is described. Similarly, a staining technique in thin smears, which has proved very satisfactory, is described.

3. In order to diagnose and treat patients with malaria properly, it is necessary to have confirmation of clinical diagnosis of malaria.

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A DEVICE FOR THE PROTECTION OF MICROTOME SECTIONS*

R. Weiss, M.Sc., Jerusalem, Palestine

BANDS of microtome sections are usually deposited on a sheet of paper as they come from the knife, before being mounted on slides. This practice exposes the sections to the risk of being disturbed or scattered by the slightest draught. For better protection of the sections I recommend the following device which has been in use in our institute for some time.

The device consists essentially of a rectangular framework containing the sheet of paper on which the bands will be deposited, and of a glass cover sliding on the framework.

A wooden board bearing on its longer sides two ledges, transverse section shown in *B* of Fig. 1, serves as a support for the sheet of paper on which the bands will be deposited. A glass cover (*3*), indicated by dotted line, with a handle (*4*) is fitted so as to slide easily on the ledges. There are two shorter ledges (*5*) at both sides of the paper flush with the lower level of the long ledges.

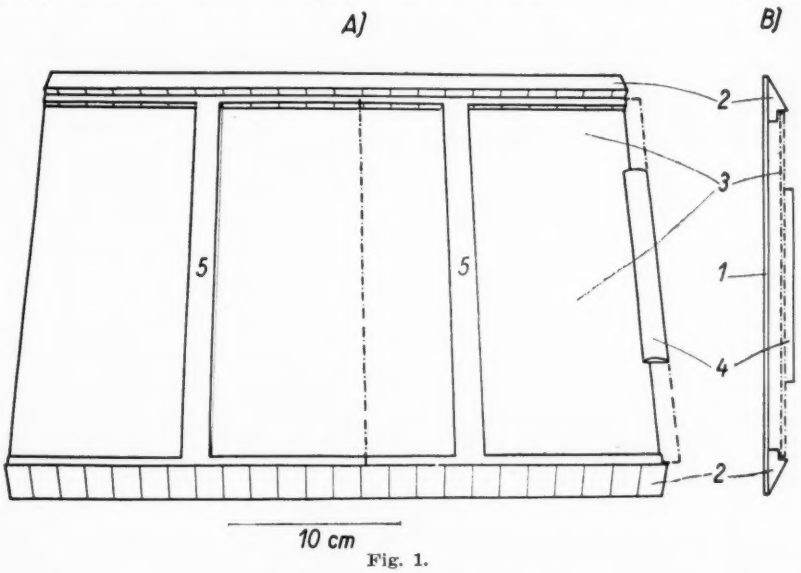

Fig. 1.

The series are deposited from the knife onto the paper in the framework. The glass cover is pulled by the handle from left to right, successively covering each new band. The framework protects the bands from any dust or draught which might come from the sides, while the glass affords protection from above. When we mount the sections we pull the glass cover to the right, successively uncovering them.

Since with this device we shift the glass instead of lifting it, we avoid eddies which might disturb the bands.

The size of the whole board was 27 × 38 cm., to fit a sheet of 14 × 22 cm. writing paper. The glass cover should slide on ledges at least 7 mm. high. The use of ready-made ledges as used for picture frames is most advisable.

*From the Department of Zoology, Hebrew University, Jerusalem, Palestine.
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A TIME AND TEMPERATURE GUIDE FOR INSPISSATING CULTURE MEDIA*

A. Hambleton, M.A., London, Canada

FOR many types of culture media which are prepared by inspissation, it is desirable to raise the temperature of the inspissator at a rather slow and steady rate in order to get a uniform product, free from holes and defects. One method to accomplish this is to make a chart of time and the corresponding temperature, and to have a clock which is turned back to the "zero hour" at the start of each inspissation.

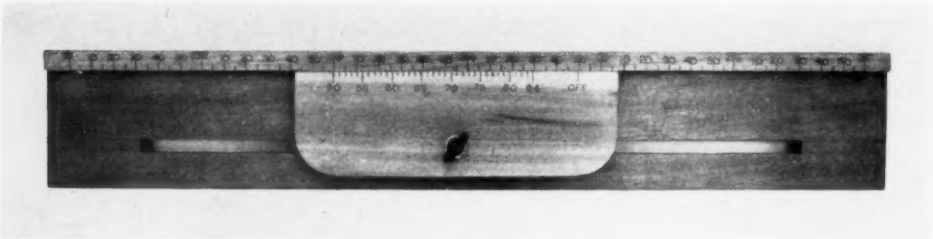

Fig. 1.

Another method, and one which we prefer, is to make a simple "slide rule," by which the temperature at the start of inspissation is set opposite the actual time of the day, and which indicates from then on the temperature which the inspissator should have at any time until the batch is complete. We use this temperature guide in making Petragnani's medium for cultivating tubercle bacilli; the temperature is raised from 50° to 84° C. during 1½ hours, and then held for twenty minutes at 84°.

The illustration shows the rule marked for this purpose. Time is marked on the long fixed scale, from 9 A.M. to 3 P.M. and again from 1 to 7 P.M. The smaller moveable part, which can be placed and held at any setting by the slot and wing nut bolt, is marked for the temperature, and the space between the 50° and 84° marks corresponds to 1½ hours. The temperature guide, as we made it, is 50 cm. long and 8 cm. wide; obviously any desired size can be used and may be graduated for any rate of temperature rise.

*From the Laboratory of Queen Alexandra Sanatorium.
Received for publication, Nov. 11, 1943.

THE REACTION OF XENOPUS LAEVIS DAUDIN (THE SOUTH AFRICAN CLAWED FROG) TO HUMAN PREGNANCY SERUM*

A New Test for Pregnancy. A Preliminary Report

Samuel S. Rosenfeld, M.D., and Vilma W. Rosenfeld, R.N., New York, N. Y.

CLOSE association with Weisman influenced us in employing the female Xenopus Frog Test for pregnancy. We followed the technique published by Weisman, Snyder, and Coates,[1-3] using a urine concentrate as described by them.

The results obtained by us with the urine concentrate were very satisfactory. However, having observed the action of human pregnancy serum in the rat and in the rabbit (Rosenfeld, Lapan, and Baron[4]), we decided to experiment on this species of frog with human pregnancy serum.

MATERIALS AND METHODS

Mature female frogs, *Xenopus laevis*, were employed. They had previously been tested by us with injections of urine concentrates from known pregnant and nonpregnant women, and the reactions were true; i.e., all the pregnant urines caused the extrusion of eggs whereas the nonpregnant urines failed to do so.

Serum was obtained in the ordinary manner by drawing blood from a vein and centrifuging the same after a short settling period. The serum was now ready for injection. This was usually done a day after the blood was obtained, but in several instances because of the lack of frogs the serum was not used for periods ranging up to four weeks.

Our first effort was to ascertain whether human pregnancy serum could cause extrusion of frog eggs. Having a store of pooled human pregnancy serum, we commenced our experiments with this material. We injected the first frog with 1 c.c. of pooled pregnancy serum, and after 48 hours, the result was negative. We repeated this experiment on another frog, this time injecting 2 c.c. of pooled pregnancy serum followed by another injection of 2 c.c. of the same, after 14 hours. The result was positive 8 hours after the last injection. We then found that single injections of 3 and even 5 c.c. of pooled pregnancy serum gave negative reactions, but additional subsequent injections of only ½ to 1 c.c. into the same animal yielded positive results.

We continued injecting the pooled pregnancy serum and found that three injections of 0.5 c.c. at 3- to 4-hour intervals yielded the best and the quickest results. Two injections of 0.5 c.c. at 3- to 4-hour intervals would also yield positive results, but the animals extruded many less eggs. Positive and satisfactory results were also obtainable with three injections of 0.25 c.c. No

*From the Department of Obstetrics and the Laboratories of the Jewish Memorial Hospital.
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positive results were obtained with amounts of less than 0.25 c.c., even when three injections were made at 3- to 4-hour intervals.

We then tested individual sera from known cases of pregnancy, usually employing three injections of 0.5 c.c. at 4-hour intervals. In a few instances the test was positive after the second injection, and on one occasion after the first. In these cases no injections were given after the test was seen to be positive.

Sera were examined from women in the first, second, and third trimesters. The tests were all positive. The earliest positive appeared 1½ hours after injection, but in the main, the time of appearance of the eggs ran parallel to that observed when urine concentrates are employed, i.e., in from 6 to 18 hours

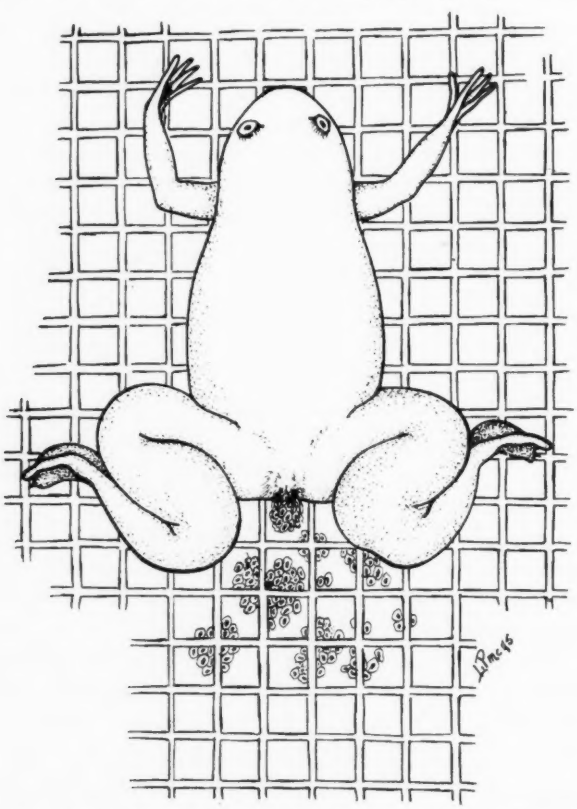

Fig. 1.—Positive test, actual drawing. Note eggs in the process of extrusion from cloaca.

For controls, male sera, definitely proved nonpregnant sera, and sera from women in the menopause were injected. All these sera gave negative results.

DISCUSSION

Hogben[5] showed that extracts of the anterior pituitary lobe caused external deposition of eggs in the hypophysectomized mature female *Xenopus laevis* (the South African clawed frog).

In 1934 both Shapiro and Zwarenstein[6] and Bellerby[7] working independently suggested the use of the Xenopus frog as a test for gonadotropic hormones in pregnancy urine.

Employment of serum has some advantages over the use of urine concentrate, and at certain times has definite indications. Specimens are easily obtained, and only a few c.c. of blood need be drawn, for 1.5 to 2 c.c. of serum is ample. After blood is drawn, it is a matter of minutes to get serum. The obtainment of the urine concentrate, however, necessitates several chemical procedures which in the best-equipped laboratories consume about three hours, and in the hands of the practitioner will take three or four times that.

Instances may arise where a test for pregnancy is clinically necessary, and yet it is desirable not to have the patient know or suspect the reason for performing the test. In such cases a serum test would arouse no suspicion, while a urine test in an intelligent woman would be almost a complete "give away."

While the time required to produce a positive reaction is about the same when either urine concentrate or serum is employed; nevertheless, since the first morning urine is usually used because of its higher hormone content, the actual or real elapsed time when serum is used is shorter because blood can be taken at any time of the day. Indeed, a positive test can be obtained by using serum before the urine specimen is even available for concentration.

Summary.—The South African clawed frog (*Xenopus laevis*) is a suitable animal for the demonstration of gonadotropic hormones. These hormones are present in the blood of pregnant women, and when injected into this species of frog cause an extrusion of eggs. The end result is macroscopic and can be read with great facility. Blood serum has several advantages, such as ease of obtainment and preparation; the "real" time for the appearance of a positive reaction is shorter, and in instances where it is desired to keep the patient from thinking that pregnancy is suspected, serum is preferable.

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A VEHICLE FOR THE INTRAVENOUS ADMINISTRATION OF FAT-SOLUBLE HORMONES*

M. H. F. Friedman, Ph.D., Philadelphia, Pa.

THE intravenous route for the administration of hormones is limited to those which are water-soluble. The fat-soluble hormones are usually restricted to some other method of parenteral injection. In experimental work it is often desirable to study the effects and the fate of fat-soluble hormones when given directly into the blood stream. This has been done by various workers by administering the hormone as a fine suspension in saline (Emery et al., 1943) or as a solution in alcohol (Cantarow et al., 1942). The danger of emboli formation in these procedures appears to be negligible when the amount of the hormone administered is small. However, the low solubility of some of the fat-soluble hormones, notably estrone, may require the use of relatively large amounts of the solvent. Unfortunately, because of their deleterious effects, the usual fat solvents cannot be administered by vein in large quantities with any great degree of safety.

Because of their properties, the suitability of the higher polyethylene glycols were studied by us, particular attention being paid to a compound known as "Carbowax 1500."† This compound is characterized by a large number of ether linkages and a high degree of chemical inertness. The molecular weight is about 1250, density $1.152^{20°}$, melting point between 30 and 33° C., and pH of 50 per cent aqueous solution 7.6 (glass electrode). At room temperature the substance is light amber in color with the consistency of petrolatum, while in the liquid state it is faintly brown and somewhat viscous. It is probably a mixture of several of the higher polyethylene glycols.

The polyethylene glycol is soluble in blood and blood serum in all proportions. Single intravenous injections of 50 mg. androsterone, 50 mg. testosterone, and 150 mg. desoxycorticosterone, each dissolved in 3 c.c. "Carbowax 1500," have been made in dogs without untoward reactions. While these hormones precipitate out from solution when the solution is added in vitro to blood serum, reactions due to intravascular precipitation appear to be negligible. The results of intravenous administration of these hormones will be presented at a later date; the present paper is a preliminary report on the toxicity of the polyglycol when given by vein.

The only toxicity studies on "Carbowax 1500" reported to date are those of Smythe et al. (1941, 1942). No microscopic abnormality of any of the organs examined was found in rats receiving the polyglycol in drinking water up to 4.05 Gm. per kilogram body weight per day for ninety days. Much larger single

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†We are indebted to Carbide and Carbon Chemical Corp., for a generous supply of the polyethylene glycols.

doses fed to albino rats, guinea pigs, and rabbits were likewise without demonstrable effects on the liver and kidneys.

We have administered "Carbowax 1500" intravenously to twelve rabbits in single doses of 1 to 2 Gm. per kilogram body weight without observing any reactions. Four male mongrel dogs were given each by vein 0.5 Gm. per kilogram of the polyglycol and no disturbance was noted in respiration, pulse, or temperature during the period of four hours following. All dogs were still in apparent good health two months later.

Three female dogs and one male dog were given each 0.75 Gm. "Carbowax 1500" per kilogram per day for three successive days and blood studies were carried out for a period of three weeks. The red and white count, hemoglobin, and hematocrit remained normal throughout this period. Three of these dogs were sacrificed one, four, and six months later without any demonstrable pathologic changes found. The fourth dog, a female, is still living and in good health twenty months later. The Carbowax injections in this dog did not interfere with the birth of five puppies six months after administration.

The effects of the polyglycol on the blood pressure was studied in nine dogs under pentobarbital anesthesia and on six unanesthetized rabbits. When administered intravenously in 50 per cent solution in distilled water, no effect was observed in the dog in doses up to 4 Gm. per kilogram. When administered undiluted, there was often a slow rise in blood pressure of about 15 mm. Hg; this returned to normal within 15 to 25 minutes. This hypertensive action was noted more frequently when higher doses (3 to 4 Gm. per kilogram) were used. Intravenous injection of 0.5 to 1.0 Gm. per kilogram of the undiluted polyglycol produced in the rabbit a transitory rise in blood pressure (measured in the central artery of the ear) of 5 to 15 mm. Hg.

These experiments do not indicate the limit of tolerance for the "Carbowax 1500" nor do they reveal the ultimate fate of the substance after administration. Better chemical tests for the identification of the substance must be developed for study of the latter problem. These preliminary results are presented at this time with the caution that further investigation is needed. We believe that not only "Carbowax 1500" but also other higher polyethylene glycols may prove suitable vehicles for the intravenous administration of fat-soluble hormones (and vitamins), at least in experimental work. The small amount of the hormones usually required to produce the desired physiologic effect and the relatively high solubility in the polyglycol are in favor of the procedure.

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CULTIVATION AND PRESERVATION OF DIPLOCOCCUS PNEUMONIAE AND MYCOBACTERIUM TUBERCULOSIS IN MARKET EGGS

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THE recent publication by Blattner, Keys, and Hartmann (Archives of Pathology **36**: 262, 1943) adds another contribution to the use of fertile eggs as culture medium for a number of delicate microorganisms. This method has come to the fore as a result of the work of Goodpasture et al., by which the chick embryo has been established as a standard medium for most of the pathogenic viruses.

It should, however, be emphasized that the value of fertile eggs as a nutrient medium for bacteria has been stated exactly one-half a century ago by Maffucci* (1894). Even earlier (1888), Hueppe† reported that "the sterile unboiled egg is an excellent medium for many microorganisms." Therefore, it seems strange that while nowadays the hatching egg has come into vogue as a medium for many pathogens (including certain spirochetes), the use of the ordinary market eggs, in spite of the obvious advantages, has not been resumed to any extent. Furthermore, it must be considered that inoculation of infective material into fertilized eggs does not result in a true cultivation of the implanted microbes; it is rather an infection of the developing embryo, which, including its membranes, is being invaded by the microorganisms, causing death of the embryo in a short time.

On the other hand, the common fresh market egg has proved to be a very suitable medium for pathogens. This is demonstrated by the following study for which we chose diplococcus pneumoniae and mycobacterium tuberculosis. The technique employed was as follows:

The natural air space end of the egg was painted with tincture of iodine, and a small hole was drilled into the shell with a thin sterile burr. A few drops of blood from a mouse infected with pneumococcus Type I and III respectively, or a few drops from a broth culture, or of a saline suspension from a plate culture were injected. After sealing the opening with sodium silicate, the eggs were incubated either at room temperature or in an electric incubator at 100° F.

It was found that the growth of pneumococci in market eggs *proceeded at room temperature to the same extent as in the incubator.* By aspirating with a sterile syringe, specimens were taken at three-day intervals from different areas of the eggs. Examination of stained smears revealed that the pneumococci in pure culture were spreading throughout the eggs.

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*Maffucci, A.: Centralbl. f. allg. Path. u. path. Anat. 5: 1, 1894.

†Hueppe, Ferd.: Centralbl. f. Bakt. 4: 80, 1888.

Transmission to animals was successful with material from eggs up to eight weeks after inoculation, without loss of virulence or type specificity. Egg material, yolk as well as white, in a dilution of 1:1,000,000, when injected intraperitoneally into mice, caused death of the animals within eighteen to twenty-four hours. The virulence of the cultivated strains, as tested in mice and rabbits, remained unaltered after repeated egg passages or prolonged cultivation in the same egg. The Neufeld capsule swelling test was applied to yolk and egg white material, washed with saline to remove adhering protein substances. The results indicated that the type specificity was maintained.

The tubercle bacillus (typus humanus) could be cultivated in the same manner without difficulties. The strain used had been recently isolated from an active case. After one transfer on Petroff's medium, culture material was inoculated into eggs. In fertile eggs, growth of the bacillus was found to be almost limited to the chorio-allantoic membrane. In ordinary market eggs, however, development of the bacilli is not confined to isolated parts but spreads throughout the egg. By this technique fair multiplication of the bacillus is being obtained on the fifth or sixth day following inoculation. This is the case whether the eggs are kept in the incubator or at room temperature.

Microscopic examination of stained smears from aspirated egg content shows bacilli arranged in irregular groups, sometimes in clumplike aggregates. By intraperitoneal injection in guinea pigs it was shown that the virulence of the cultivated bacilli did not differ from that of the original culture.

These observations prove that the ordinary fresh market egg is a suitable and valuable medium for the cultivation and preservation of pneumococci and tubercle bacilli. It can just as well be utilized for the primary isolation of these microorganisms and probably for others as well. It is noteworthy that the author did not encounter any difficulty because of nonsterile eggs or secondary contamination. There is no need for using fertilized eggs. The normal market egg offers the advantage that it is by far the cheapest and simplest nutrient medium; it is easily obtainable and can be kept at room temperature. By using this method, the physician with restricted facilities is enabled to cultivate bacterial agents from suspected material (sputum, body fluids, excretions) and to perform the "typing" in cases of pneumonia. In doubtful tuberculous infections, cultivation of the Koch bacillus in eggs may prove an acceptable substitute for the animal experiment.

ON THE USE OF MALLORY'S PHOSPHOTUNGSTIC ACID HEMATOXYLIN FOR STAINING INTESTINAL PROTOZOA*

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AT THE present time, perhaps as never before, it is essential that diagnostic laboratories be prepared to make accurate fecal examinations for intestinal protozoa. A major handicap to such examinations, and in particular to identification of *Endamoeba histolytica*, is the nature of the staining routines usually recommended for preparation of permanent slides.

The methods most commonly outlined in books on laboratory procedure require the use of Heidenhain's iron hematoxylin or one of its many modifications. Most of these involve overstaining of the specimen, to be followed by destaining. Because of the size of the organisms to be identified, differential destaining must be followed with the microscope and at high magnification. Except in experienced hands this process is uncertain and prolonged.

Procedures by which differential destaining may be omitted, either by carefully timed staining periods[1] or by acidifying the stain,[2] have not given satisfactory preparations in our experience.

A stain that seems to meet the needs of routine examinations rather completely is Mallory's phosphotungstic acid hematoxylin, used as indicated here. The original description[3] of the procedure by which this stain was applied to fecal smears does not give entirely satisfactory results: first, apparently because fixation time is too short; second, because the fixative advocated does not contain acetic acid; and third, because the staining period cannot be stated exactly.

In our hands the following schedule has been tested many times and has given satisfactory preparations regularly. Nuclear stains have permitted ready identification of intestinal amoebae and flagellates, although structural details may not be quite so sharply defined as with the best of iron hematoxylin preparations.

1. Prepare fecal smears in the usual way and fix in Schaudinn's fluid or in any of its modifications, which contain about 5 per cent acetic acid; 1 to 24 hours. (Bouin's fluid has not given useful preparations.)

2. Remove bichloride of mercury from smears in the usual way.

3. Wash in 70 per cent alcohol to remove iodine.

4. Wash in water.

5. Stain in Mallory's phosphotungstic acid hematoxylin for 20 to 30 minutes. The time interval will vary with the age of the stain or its degree of "ripeness" and must be determined by trial.

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6. Wash in running water for five minutes or until the smears become blue.

7. Dehydrate slowly in 95 per cent and absolute alcohols. Otherwise smears may appear cloudy when examined with oil immersion lens.

Cytoplasm of the trophozoites of amoebae and flagellates appears pale blue and the nuclei darker blue, but within cysts, nuclei are much more clearly stained than usual, chiefly because the cytoplasm is so lightly colored. In smears containing cysts and trophozoites of several species of amoebae the larger specimens stain more deeply but all can be identified.

It is to be remembered, however, that no staining method can compensate for degenerative changes within nuclei of cysts and trophozoites of intestinal protozoa when stool specimens are held too long at room temperatures before smears are prepared. That such changes occur rapidly is understood too infrequently.

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LABORATORY SUPPORTS*

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IT IS not uncommon to find that the supports (ring stands) which are available in the laboratory are either of the wrong size or are found to be too light in weight, a fact especially noticeable in the performance of microanalytical work. We found that supports with various bases can be simply and inexpensively made in the laboratory. The bases are cast from melted scrap lead, using a plaster of Paris matrix or a discarded tin can as a mold. Scrap lead can be purchased for a few cents a pound from a junk dealer; the price of new lead is about eight cents.

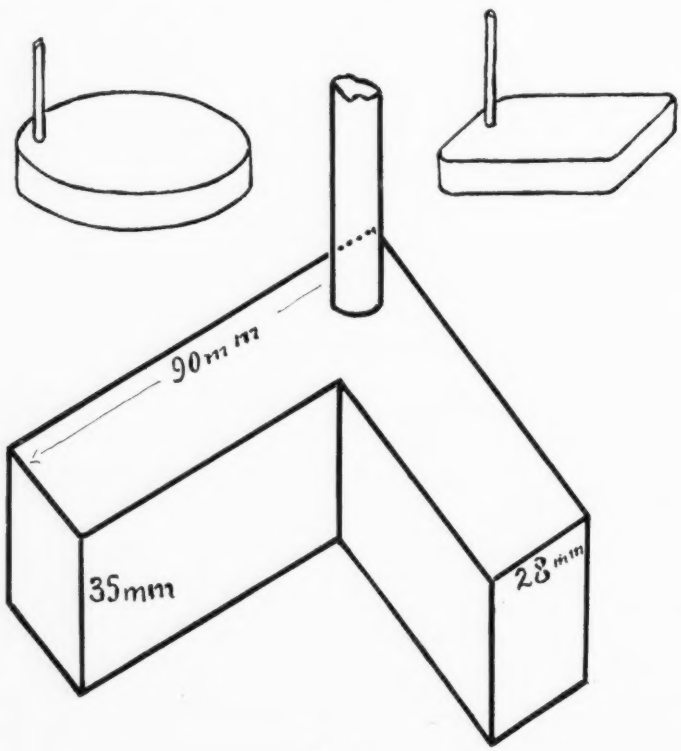

Fig. 1.

The "pattern" is made by nailing or gluing pieces of wood together. The wood must be oiled or painted. The mold is cast in a strong pasteboard box (or cigar box), oiled or painted with paraffin. A layer of plaster of Paris about

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one-half inch high is poured into the box first; the wood model is placed on top of this layer, and the rest of the plaster is poured around the pattern. After the mold has hardened, the wood is removed. It is advisable to drive two or three nails into the pattern to facilitate removal. The mold is dried in an oven or by any other suitable method.

Before the lead is poured, the support rod is put in position in the matrix, holding it fairly rigid and exactly vertical in a burette clamp. The rod should be notched with a file or a chisel in a number of places for better contact. We find that a so-called brass-plated curtain rod ($^5/_{16}$ inch) serves very well for smaller stands. Short lengths of small pipe can also be used. The lead is melted, the dross is removed from the surface, and the metal is poured into the form. If the pattern was smooth, the stand can easily be removed and the mold used again.

Round and oblong small bases can be cast by using flat tin cans as molds. If the can is coated with library paste and allowed to dry, the lead will, as a rule, not stick to the form. Fig. 1 illustrates such stands. The L-shaped support ($28 \times 35 \times 90$ mm.) weighed about three pounds. If desired, the corners can be rounded and the assembly painted.

BLOOD AGAR PLATES*

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ONE of the inconvenient problems of the small clinical laboratory is the question of a constant supply of sterile blood for blood agar plates commonly used especially for cultures of streptococci, pneumococci, gonococci, and for the hemophilic bacteria. This ordinarily involves preparation of glassware, defibrinating agents, and sterilization of syringes, not to mention the keeping and feeding of animals or the search for a willing human donor.

Practically all hospital or clinical laboratories receive blood specimens daily for serologic examinations. These are naturally collected aseptically and, if from local source, quickly reach the laboratory in an essentially sterile condition.

For many years we have followed the procedure of breaking loose the clot with a sterile loop, then placing these tubes in the refrigerator. When the clot has retracted, we draw off aseptically only the minimum amount of serum required for the given test. The tube containing the clot and residual serum is held in the refrigerator at about 6 to 7° C.

As the demand arises, we rotate such a tube of blood clot and serum rapidly between the hands. The serum takes up the free corpuscles and commonly yields sufficient dilute blood to make one or sometimes two blood agar plates rich enough in color and in serum to serve excellently both for enrichment and for detection of *alpha* or *beta* hemolysis.

If the blood is collected and handled aseptically, we have rarely a contamination in such plates. Since they are poured immediately before streaking, an occasional isolated contaminating colony is of little importance. It need scarcely be emphasized that these plates must be freshly prepared, lest minute colonies develop on standing and be distributed by the loop if inoculated on the following days.

It is well also to date the tubes and to use the most recent acquisition, and constantly to replace the older unused tubes with fresh stock.

By this simple expedient we have for years evaded the inconvenience commonly associated with this problem.

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THE EFFECT OF THE DISTAL APPLICATION OF A SPHYGMOMANOMETER CUFF ON LOCALIZED VENOUS PRESSURE AND AS AN AID IN VENIPUNCTURE

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TO FACILITATE venipuncture, those expedients which result in a rise of venous pressure around the site of election are employed. Gunther[1] has stressed the role of intramuscular pressure in the maintenance of venous return flow and illustrates his point by citing the practically utilized turgidity of the anticubital veins of the proximally tourniqueted arm when the fist is clenched and the muscles of the forearm are thereby put under increased tension. He attributes the positive venous pressure in the extremities to a reflected positive intramuscular pressure. The author is inclined to believe that a major portion of the positive venous pressure in the superficial veins of the extremities is a reflection of the positive capillary pressure of the skin, citing, in this connection, that anticubital venipuncture is much more easily performed when the skin capillaries are not constricted by cold or by shocklike states.[2]

It is common knowledge among the physicians and nurses who staff the various blood donor centers that constriction around the wrist or pressure upon the flexor surface of the forearm will considerably augment the flow of blood from the site of the anticubital venipuncture. This phenomenon cannot be due, exclusively, to a "milking out" of stagnant blood in the forearm. In a series of experiments designed to elucidate the mechanism of this augmentation, it was found that steady pressure brought about by inflation of a blood pressure cuff, applied around the wrist, to an extent above that of the mean arterial pressure would, in most instances, result in a trebling of the rate of venous blood flow. Where the venipuncture had not already been performed, the superficial veins would become very prominent, turgid, and easily entered by the needle. In obese subjects the median cephalic vein, ordinarily undetectable after the usual application of the tourniquet, becomes easily distinguishable by palpation from the surrounding (and particularly in females, the frequently overlying) muscle and thereby becomes the vein of election over the median basilic for phlebotomy.

Fig. 1 illustrates the procedure. The cuff on the wrist is first applied; then the tourniquet, which consists of pliable rubber tubing about 1 inch in diameter, is tied *tightly* around the upper arm being secured by a single looped slip knot for ready release. The cuff is then inflated to from 100 to 120 mm. Hg. Either of the main anticubital or one of the superficial dermal veins will be easily accessible in over 99 per cent of subjects, however obese. Since it is undesirable to protract the double constriction, antiseptic preparations had preferably been made over a wide area around the anticubital fossa, beforehand.

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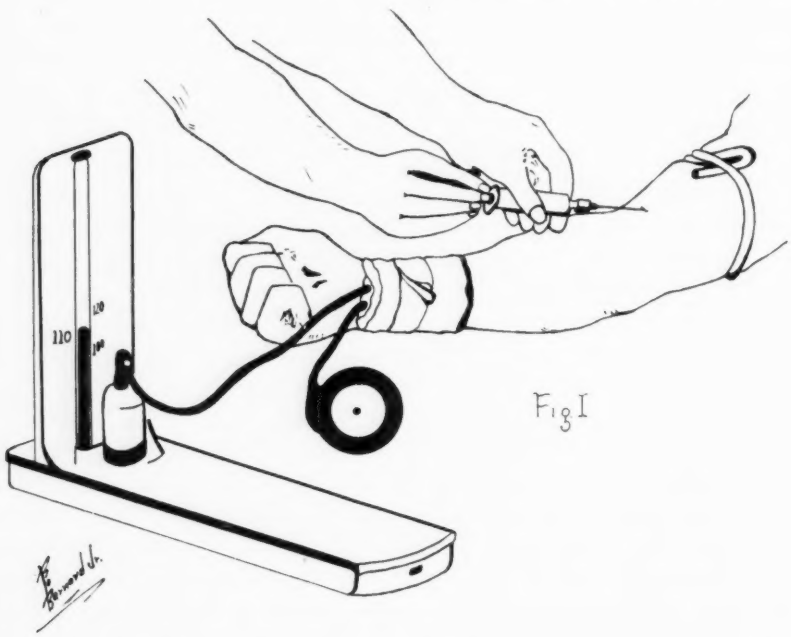
The procedure is not recommended for unmasking the veins of subjects in shock for the purpose of infusion. In fact, it should not be employed except on relatively sound individuals for the securing of blood for testing or for transfusion. These considerations arise from certain observations made in connection with its use. In the first place there is the possibility that the increased venous pressure is brought about by an increased capillary flow, the latter in turn being a reaction to trauma in the form of localized anoxia. For, if ordinary venous stasis from the proximal tourniquet were the only operative factor, it would be necessary to have the latter only sufficiently tight to exert a pressure slightly above that ordinarily extant in the veins of the extremities; in no physiologic instance greater than 20 mm. Hg. However, as in recent ordinary practice, there is substituted for the proximal tourniquet, a sphygmomanometer cuff; the latter must be inflated to above the diastolic arterial pressure before pronounced augmentation of the venous pressure, distal to it, results. In the described method, the proximal tourniquet is tied correspondingly tightly.



Secondly, the blood collected by this procedure shows a departure from the physiologic clotting sequence. This may be noticed in subjects in whom attempted venipuncture has caused injury to the vessel wall with the resultant formation of a hematoma. Usually such extravasation collapses the vein so that blood cannot be secured; if a flow does result, then clotting induced by extrahematic thrombokinase will shortly terminate it. These limitations do not hold with the tight proximal tourniquet. It is possible to continue the collection of blood in the presence of a large hematoma by the application of the described procedure, even if the needle is not within the vein and provided its lumen is not occluded, the supply being maintained from the hematoma, itself, which

constitutes the reservoir fed from the rent in the vein wall and in which reservoir the blood remains fluid much longer than would be the case under ordinary circumstances. This fact is cited, not in advocacy of the practice of collection of blood from hematomas (which may, however, be a useful expedient where the particular blood sample must be obtained), but to point out that there may be a physiologic alteration in the blood so collected and that the procedure is called for only in those instances where the ordinary ones do not suffice.

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CHEMICAL

THE RELATION BETWEEN THE PANDY TEST AND TOTAL PROTEIN CONTENT OF SPINAL FLUID*

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SINCE the reports in the literature regarding the relationship between the Pandy test and the total protein in the spinal fluid are contradictory, and since it is the policy in some hospitals not to perform a quantitative protein estimation on the spinal fluid in the presence of a negative Pandy test, we decided to determine the relationship between the two.

Table I

	TOTAL	BELOW 50 MG. NEG. PANDY	BELOW 50 MG. POS.	ABOVE 50 MG. NEG. PANDY	ABOVE 50 MG. POS.	ABOVE 100 MG. NEG. PANDY	ABOVE 100 MG. POS.
Brain tumor	153	48	5	46	54	0	20
Spinal tumor	55	19	1	14	21	0	13
Convulsive state	36	31	0	5	0	0	0
Vascular Disease C.N.S.	160	104	6	36	14	0	2
Degenerative Dis. C.N.S.	285	222	11	41	11	0	5
Infectious Dis. C.N.S. (except syphilis)	69	33	4	9	23	1	9
Traumatic Encephalopathy	25	19	1	5	0	0	0
Functional Nervous Disease	24	16	2	5	1	0	0
Peripheral Neuritis	33	17	0	9	7	0	4
Muscular Dystrophies	15	14	1	0	0	0	0
Syphilis	86						
Paresis		1	1	3	10	1	2
Tabes		14	1	6	1	1	0
C.N.S. Lues		24	4	12	9	1	3
Miscellaneous	59	38	1	14	6	9	4
Neurofibromatosis							
Arthritis							
Dysendocrinism							
Optic Atrophy—Unknown etiology							
Migraine							
Cerebral Agenesis, etc.							

The records of 1,000 neurologic admissions were examined. The nature of the clinical material used in this study is shown in Table I. Ling's[8] method was used for the protein determinations. It gives comparable results to the other procedures employed in estimating the total protein content. Although 37.9 mg. per 100 c.c. is given as the highest range of normal protein by this method, we consider values below 50 mg. per 100 c.c. as still being within normal limits. Table II indicates the distribution of the cases according to protein content and the results with the Pandy test. Table III summarizes our findings. It is interesting to note that 57 per cent of the cases had a negative Pandy

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reaction and a total protein of 50 mg. per 100 c.c. or over. Above 100 mg. per 100 c.c., 17 per cent had a negative Pandy. The protein level at which the Pandy reaction became positive in more than 50 per cent of the cases was 70 to 75 mg. per 100 c.c.

A survey of the literature shows that many authors are of the opinion that a negative Pandy reaction definitely implies physiologic concentrations of protein and that consequently a negative Pandy reaction does not occur with pathologically increased protein concentration. Among the investigators who hold this view are Zaloziecki,[14] Greenfield,[3] and Eskuchen.[2] Liebold,[7] on the other hand, states that a positive Pandy may be found with a protein content between 16 to 30 mg. per 100 c.c. Izikowitz,[4] after studying 618 cases, concluded that negative Pandy reactions do not exclude the possibility of pathologically increased total protein and globulin concentration values. Positive reactions are no proof of pathologic protein increase nor do negative reactions constitute proof of physiologic protein relations. Neel[10] states that a quan-

TABLE II

PROTEIN CONTENT	TOTAL NUMBER CASES	NUMBER WITH POSITIVE PANDY	PER CENT WITH POSITIVE PANDY	NUMBER WITH NEGATIVE PANDY	PER CENT WITH NEGATIVE PANDY
5- 9.9	2			2	100
10- 14.9	4			4	100
15- 19.9	21	2	10	19	90
20- 24.9	61	1	2	60	98
25- 29.9	126	2	2	124	98
30- 34.9	142	6	6	136	94
35- 39.9	109	9	10	100	90
40- 44.9	84	7	9	77	91
45- 49.9	89	11	14	78	86
50- 54.9	68	9	14	59	86
55- 59.9	64	12	19	52	81
60- 64.9	50	17	34	33	66
65- 69.9	53	23	44	30	56
70- 74.9	19	10	53	9	47
75- 79.9	19	13	68	6	32
80- 84.9	4	3	75	1	25
85- 89.9	2	1	50	1	50
90- 94.9	3	3	100		
95- 99.9	7	6	86	1	14
100-199.9	50	37	74	13	26
200-299.9	9	9	100		
300-499.9	11	11	100		
500-1935	5	5	100		

TABLE III

THE RELATION BETWEEN THE PANDY AND TOTAL PROTEIN CONTENT OF SPINAL FLUID

	NUMBER CASES	PER CENT		NUMBER CASES	PER CENT
Total number with Positive Pandy	195	19.5	Total number with Negative Pandy	805	80.5
Number with Positive Pandy and Protein Below 50 mg. per 100 c.c.	38	6.0	Number with Negative Pandy and Protein Below 50 mg. per 100 c.c.	600	94.0
Number with Positive Pandy and Protein Above 50 mg. per 100 c.c.	157	43.0	Number with Negative Pandy and Protein Above 50 mg. per 100 c.c.	205	57.0
Number with Positive Pandy and Protein Above 100 mg. per 100 c.c.	62	83.0	Number with Negative Pandy and Protein Above 100 mg. per 100 c.c.	13	17.0

titative protein determination cannot be dispensed with even if the Pandy reaction is negative. Merritt and Fremont-Smith[9] write that the Pandy test is useful in indicating the presence of increased protein. However, they state that the "performance of a qualitative test does not give an accurate estimation of the degree of increase in the protein content." Quantitative tests should also be done. Dickson[1] noted that in infectious disease an increase in globulin can be detected when the total protein is as low as 30 mg. per 100 c.c.

The Pandy test is considered to be an index of the globulin fraction of the spinal fluid (Kafka,[5] Levinson[6]). In his paper, however, Pandy[11] stated that the test is not only for globulin but also for albumin. Schmitt,[13] and Merritt and Fremont-Smith[9] also believe it is not a definite test for globulin. Pappenheim[12] writes that with a negative Pandy reaction the total albumin content of the spinal fluid is always low and that a negative reaction excludes with certainty a pathologic increase of albumin. This, he maintains, holds except for isolated instances. An explanation for the negative Pandy with increased total protein may, therefore, be an increase of one or other of the fractions. Further work will evidently have to be done to clarify the relations of the globulin and albumin fractions to the Pandy test.

Our results as indicated confirm those investigators who maintain that the total protein may be increased despite a negative Pandy reaction. The level of protein at which more than 50 per cent of the cases in our series began to show a positive Pandy was 70 to 75 mg. per 100 c.c. We also found cases in which a positive Pandy was demonstrated with total protein content below 50 mg. per 100 c.c. and even as low as 20 mg. per 100 c.c.

SUMMARY

1. The records of 1,000 neurologic admissions were examined as to the relation between the Pandy test and the total protein content of the spinal fluid.

2. Of the cases, 59 per cent had a negative Pandy with a total protein content of 50 mg. per 100 c.c. or over.

3. Of the cases with a total protein content of 100 mg. per 100 c.c. or over, 83 per cent had a positive Pandy reaction.

4. The level of protein at which more than 50 per cent of the cases began to show a positive Pandy was 70 to 75 mg. per 100 c.c.

5. Our results indicate that a negative Pandy may be present with a total protein above 50 mg. per 100 c.c.

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MEDICAL ILLUSTRATION

REPAIR OF CRANIAL DEFECTS BY CAST CHIP-BONE GRAFTS

A Preliminary Report

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THE restoration of the contour in cranial and frontal bone defects was achieved during the first World War by one of three methods:

1. Costal cartilage grafts (Morestin[1]) were used to fill out the defect by a cartilaginous and fibrous tissue network.

2. Foreign materials, such as celluloid and silver plates, were employed. Many of these were later removed following foreign body reactions.

3. Osteoperiosteal grafts taken from the tibia were used extensively and successfully by Delangeniere.[2]

A later technique employed for the filling in of relatively small defects consisted in the removal of the adjacent outer table of a portion of the skull. Bone obtained in this manner was transferred to the defective area. More recently bone for large grafts has been obtained from the inner table of the ilium. However, because of the difficulty in obtaining sufficiently large bone grafts for extensive defects and of giving suitable curvature and shaping to the bone, interest in the utilization of nonirritating foreign materials was revived. The materials employed for this purpose are vitallium, tantalum, ticonium, and acrylic resins. Vitallium requires preliminary casting, a serious inconvenience. On the other hand, tantalum and ticonium can be applied in sheets that are easy to shape. These metals are difficult to obtain and expensive, however, aside from the disadvantage of always remaining foreign bodies. The tolerance of the tissues to acrylic resins has not as yet been fully ascertained.

In twelve cases recently operated on by Converse at the French Maxillo Facial and Plastic Surgery Center in Algiers consistently good results were noted in the repair of mandibular defects caused by war injuries. Chip-bone grafts removed from the ilium were used. As a result of this experience, the following technique has been evolved for the filling in of cranial defects with cast or formed masses of bone chips. This molding and casting technique has been used with excellent immediate results in three cases. Because of the extensive use made of molds and casts, their preparation is described in detail.

As a means of simplifying this text the following definitions are given. A *mold* is an impression of an original object. A *cast* is an impression from the

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mold. Accordingly, the mold is the *negative* and the cast is the *positive*. The cases described are repaired by incorporating with the bone surrounding the skull defect a cast area of bone chips that is of the desired thickness and shape to replace the lost contour and to give mechanical protection to the brain. In other words, a cast of bone chips is suitably shaped to restore the defective skull.

PREPARING THE MOLD

In preparing the metal mold for the bone chips the first step is to make an agar mold of the entire face of the patient. This procedure has been described by Clarke and Cone[3] and by Clarke.[4] From the agar mold is made a plaster of Paris cast as seen in Fig. 1. This too has been described in detail by Clarke.[5] It is of considerable advantage to make a cast of the entire face including the ears; the normal side then can be used as a guide for the restitution of the contour of the defective side.

In the case illustrated, the cerebrospinal fluid causes the skin over the defect to recede and expand. Restoration of the skull should aim at a replacement of the outer table to its proper level; also, it should aim at restoration of the normal curvature of the inner table to allow for expansion of the fluid. The plasteline is modeled into this area on the plaster cast and serves the purpose of fixing the size of the cavity, as well as affording support for the wax modeling which is to follow.

The amount of wax to be placed over this area will represent the thickness of the skull. This must be determined by considering sectional views of normal skulls.

It must be realized that this work is done over the skin surface as represented on the mold. For this reason it is necessary to consider the sectional view of the progressive work (Fig. 2). The top illustration shows the sectional view of the head with the wax and plasteline in place. The points *A* and *B* represent the area on which the bone graft eventually will rest on the natural bone of the skull. The skin flap will be turned back and the "bone plastic" put in place against the denuded surfaces of the bone of the skull. In this manner the skin shifts position to the outside of the bone plastic, as seen in the bottom part of Fig. 2, instead of lying on the inner side of the plasteline and wax reconstruction as seen in the top part of the same illustration.

After the plasteline has been put in place, as seen in the top part of Fig. 3, to represent the contour of the brain, a layer of modeling wax is applied to represent the thickness of the chip-bone graft. This wax is seen in the bottom half of Fig. 3. It is modeled so as to restore normal contours of the head. When both sides are symmetrical the modeling is completed.

A wax composition suitable for the purpose may be compounded from the following formula:

	Parts by weight
Paraffin	2
Resin (colophony)	2
Petroleum jelly	1

The paraffin and petroleum jelly are melted together. Then the resin is added and the mixture is heated slowly until the entire mass is melted. In

Fig. 1.—*A* and *B*, Case 3, Lt. B. Injury sustained from shell fragment. Skull defect 15 × 9 cm.

C and *D*, The space to represent the inner table of the cranium is outlined on the plaster cast.

summer the petroleum jelly can be reduced or eliminated. In cold weather it may be necessary to increase the amount of petroleum jelly to have the material sufficiently plastic to work as a stiff clay between the fingers. This mixture becomes fairly hard on cooling.

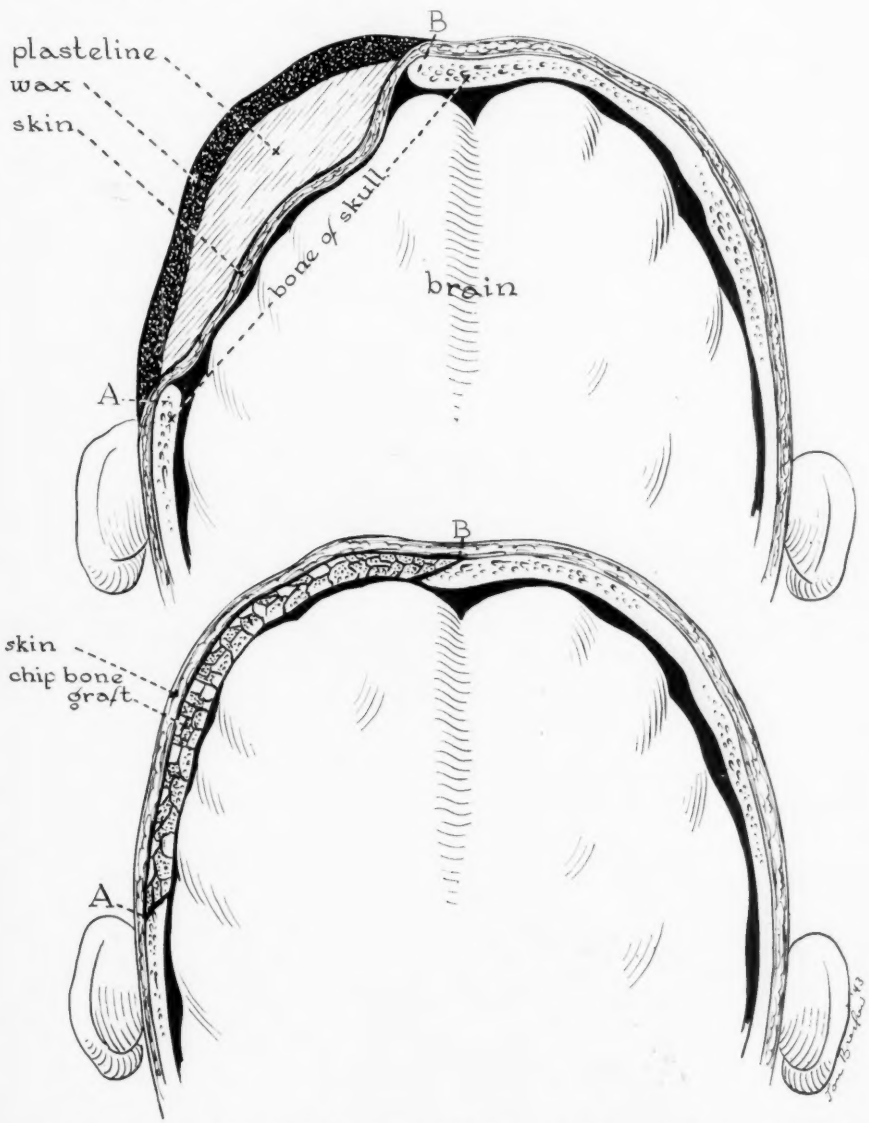

Fig. 2.—Sectional views of the head showing the wax and plasteline reconstruction and the final cast chip-bone graft. The points *A* and *B* represent the places where the chip-bone graft comes into contact with the normal bone.

When the modeling is completed and the wax mixture has cooled, a plaster mold is made over the modeled area as seen in Fig. 4*A*. This plaster mold is rubbed with talc, a separating medium, and then placed in French casting sand, Fig. 4*B*. The sand is patted flat around the mold to form an edge. The edge of the sand mold is dusted with talc to form a separation. A tin wall, Fig. 4*C*, is placed over the plaster mold. French casting sand is then put into

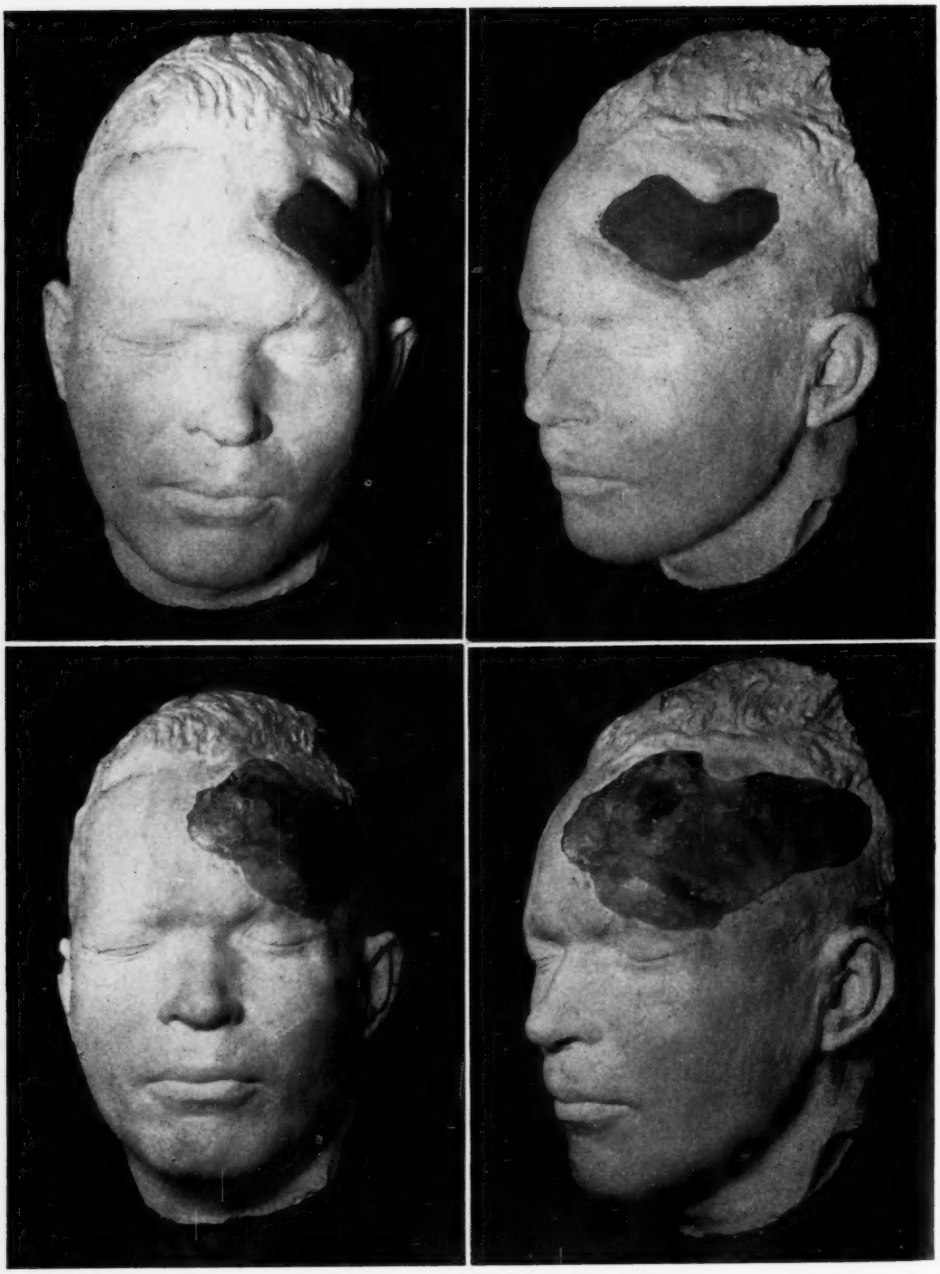

Fig. 3.—*A* and *B*, Plasteline is used to fill in the area that will represent the inner table of the cranium.

C and *D*, The wax is used to reconstruct the contours of the head. This wax will also represent the shape and thickness of the cast bone graft.

the wall and packed against the plaster mold and the sides of the wall. It is finally packed tightly with a hammer as seen in Fig. 4*D*.

The *tin wall* containing the sand and plaster mold is then turned upside down and the plaster mold is removed as seen in Fig. 5*A*. The resulting half of the sand mold thus obtained is placed on a gas burner and baked hard and dry (Fig. 5*B*). It can then be poured with lead as shown in Fig. 5*C*. One-half of the metal mold that will be used for the bone chips is thus produced.

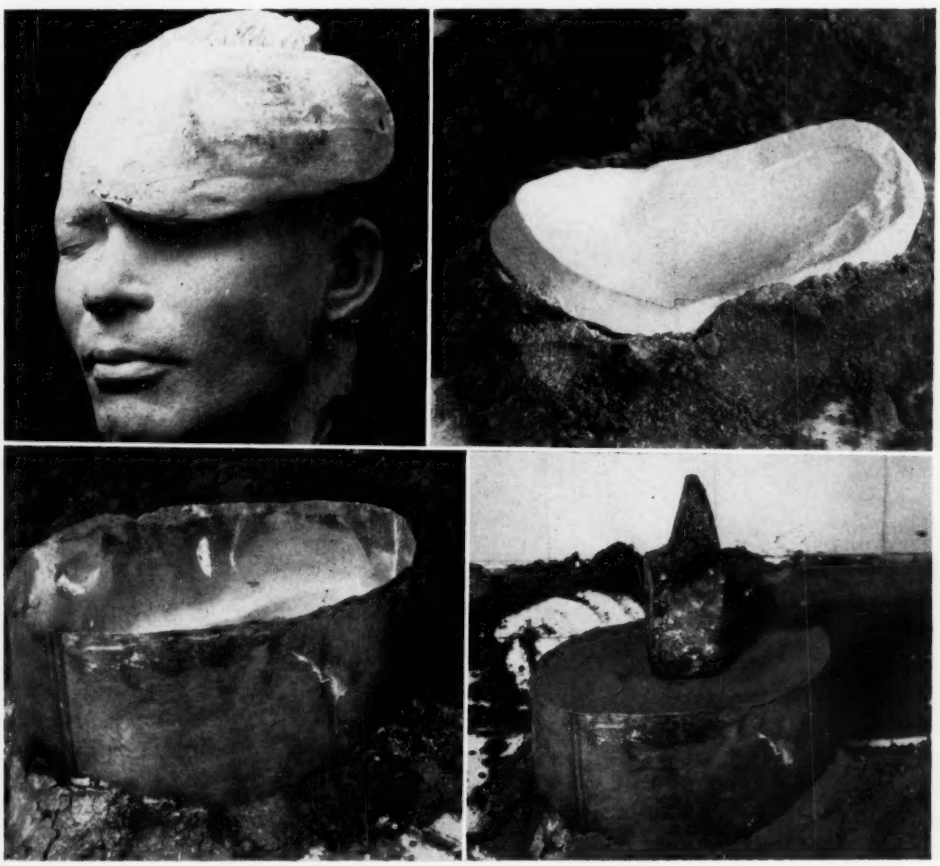

Fig. 4.—*A*, A plaster mold is made over the wax reconstruction.
B, The mold is removed and placed in French casting sand.
C, A tin wall is placed over the plaster mold.
D, The wall is filled with sand and tightly packed.

At this point it becomes necessary to remove the modeled wax piece from the plaster cast of the patient's head. If the head has been kept wet during the modeling, the wax will not stick to the plaster but will separate easily in one piece. This wax piece is imbedded in a two-piece plaster-silica mold as seen in Fig. 6. Such a mold can be made by mixing equal parts of plaster of Paris and either river sand or powdered silica while in a dry state. This is added to a predetermined amount of water as in the regular procedure of plaster casting and the mold is made. The resulting mold is opened and the wax pattern removed. The plaster-silica mold is closed and completely dried over a

gas burner. It is then tied together with wire, and melted lead is poured in through a funnel-like opening. When the lead has set and cooled, the plaster-silica mold is opened and the lead piece is removed. This will be a lead replica of the wax-modeled area and should fit the plaster cast of the patient's head in the same manner as the wax.

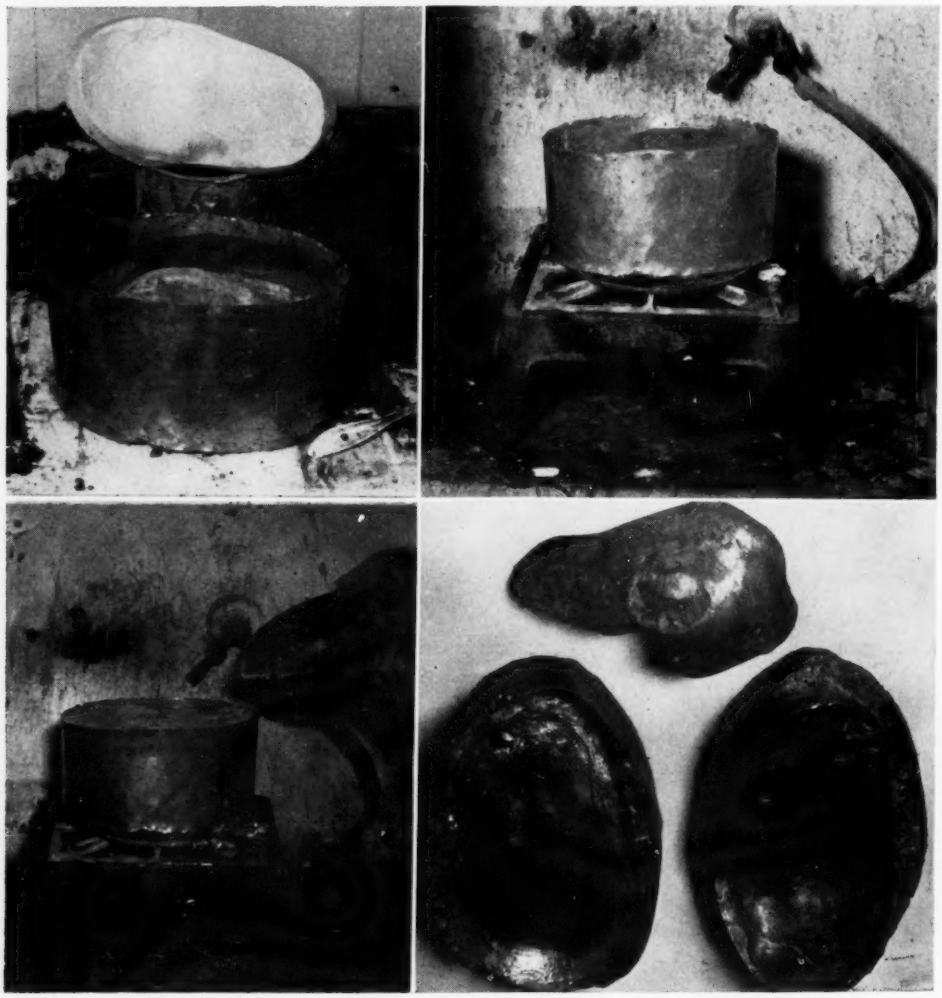

Fig. 5.—*A*, The sand mold is turned upside down and the plaster cast is removed.
B, The sand mold is baked hard and dry.
C, The sand mold is poured with metal.
D, Both pieces of the metal mold and the metal replica of the wax reconstruction.

This piece is now placed in the half of the lead mold which was prepared previously and should still be in the tin wall. A thin coating of plaster, water, and glue is painted over these lead pieces to facilitate separation of one metal part from another when the second half of the lead mold is poured. The glue in the mixture serves to keep the plaster in this separating medium from setting and helps to hold it as a thin "coat of paint" to the set metal. The second half of the lead mold is then made. It is important to remember that the lead

must not be so hot as to melt the first half of the mold; however, it must be hot enough to flow freely. When the second half has been poured and has cooled, the mold is opened, thus producing two halves of the mold and the metal replica of the wax replacement as shown in Fig. 5*D*. This is the mold which will be used to receive the bone chips. The metal replica of the wax modeling is of no further use and may be discarded. Fig. 7 is a mechanical drawing of a cross section of the metal mold or die and counterdie that is used to compress the bone chips.

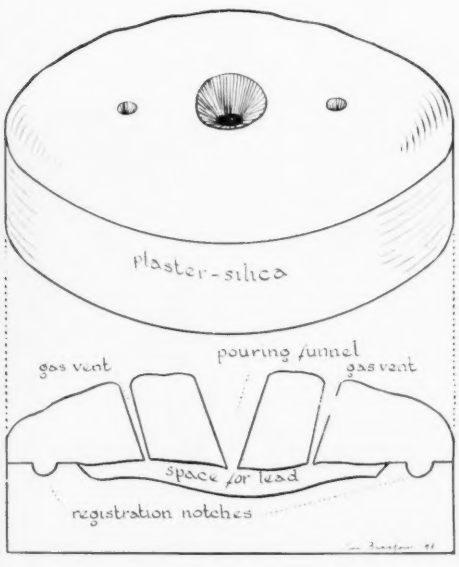

Fig. 6.

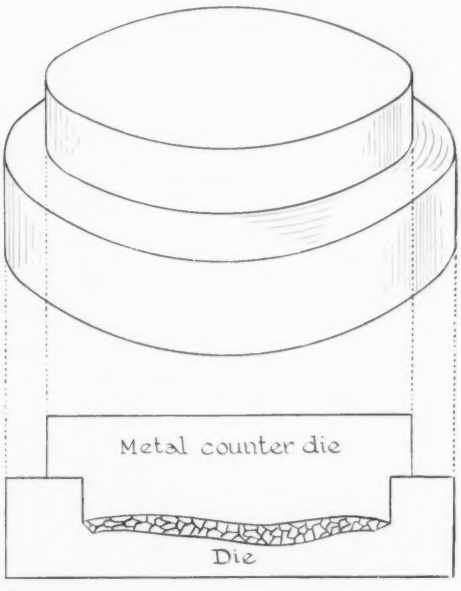

Fig. 7.

Fig. 6.—The plaster silica mold for making metal replica of wax reconstruction of chip-bone graft.

Fig. 7.—The metal mold for making the cast chip-bone graft.

THE SURGICAL PROCEDURE

The steps of the surgical procedure are: under general anesthesia (intratracheal, gas, oxygen, and ether), and after a peripheral infiltration of one per cent novocain and adrenalin, a wide scalp flap is outlined with pen and ink and incised. This flap is carefully dissected from the dura, thus widely exposing the defect (Fig. 8). The edges of the bone defect are resected in a beveled manner and the defect is then ready to receive the bone graft.

The inner table of the ilium is exposed by elevating the periosteum from the crest (top illustration in Fig. 9). A segment of the inner table is removed and sectioned into cube fragments measuring 0.25 to 1 cm. These bone fragments are packed into the lower half of the sterilized metal mold (Fig. 9*B*). The bone fragments are added to the mold to a height above that of the desired thickness of the final bone graft to insure impaction of the fragments after they have been compressed. Blood from the iliac wound is then withdrawn in a syringe and mixed with the bone fragments in the mold. The upper half

Fig. 8.—*A*, This illustration shows an extensive defect over the frontal lobe. The skin flap has been dissected away from the dura.

B, The bone edges have been beveled and made ready to receive the graft.

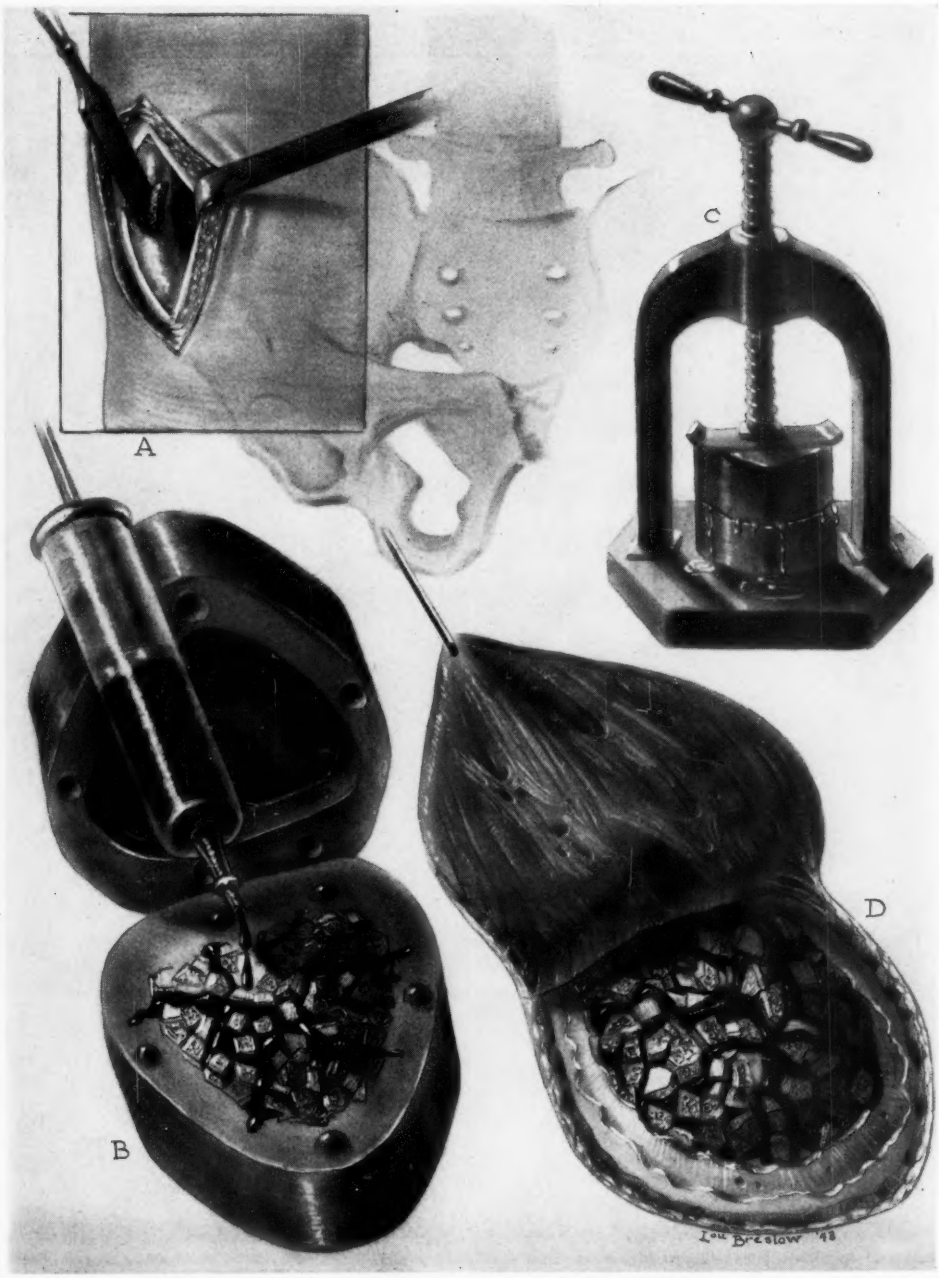

Fig. 9.—*A*, This illustration shows the removal of bone chips from the inner table of the ilium.

B, The bone chips are packed into the mold. Blood is poured over the chips.

C, The upper mold is put into position and the cast bone graft is made in a dental or letter press by the joint action of compression and coagulation of the blood.

D, The cast bone graft is put in place and the flap is ready to be reapplied.

Fig. 10.—Case 1, Sgt. G. An injury sustained in the bombing of a train. The anterior and posterior walls of the left frontal sinus were destroyed, leaving an opening 6 × 3.5 cm.

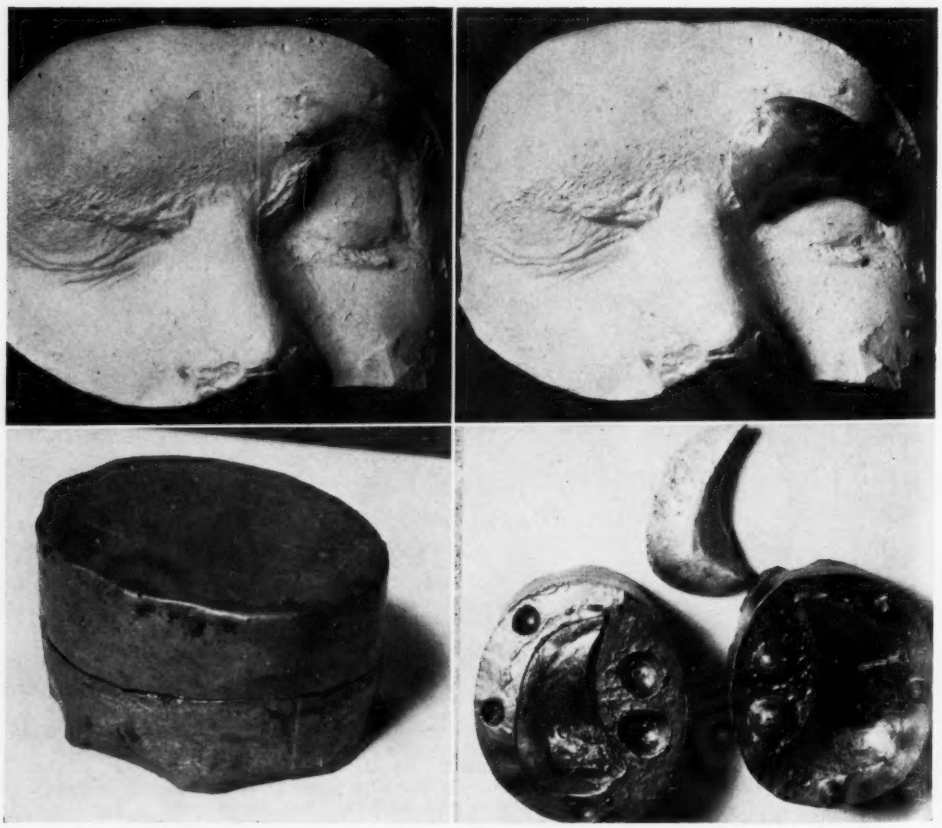

Fig. 11.—*A*, Case 1. A plaster cast of the patient's left frontal region showing the defect.
B, A plaster cast showing the metal reconstruction.
C, The closed metal mold for compressing the bone chips.
D, The open metal mold with the metal cast. This replica was used only in making the mold and was finally discarded.

of the mold is put in place and the entire mold is inserted into a sterilized strong dental or letter press (Fig. 9*C*). Pressure is applied and maintained until the blood has clotted, the fibrin of the clot acting as a cement in holding together the bone particles under pressure. This bone "plastic," shaped, cast, and held in one mass by the double action of compression and fibrin cement, is then introduced into the position covering the defect of the skull (Fig. 9*D*).

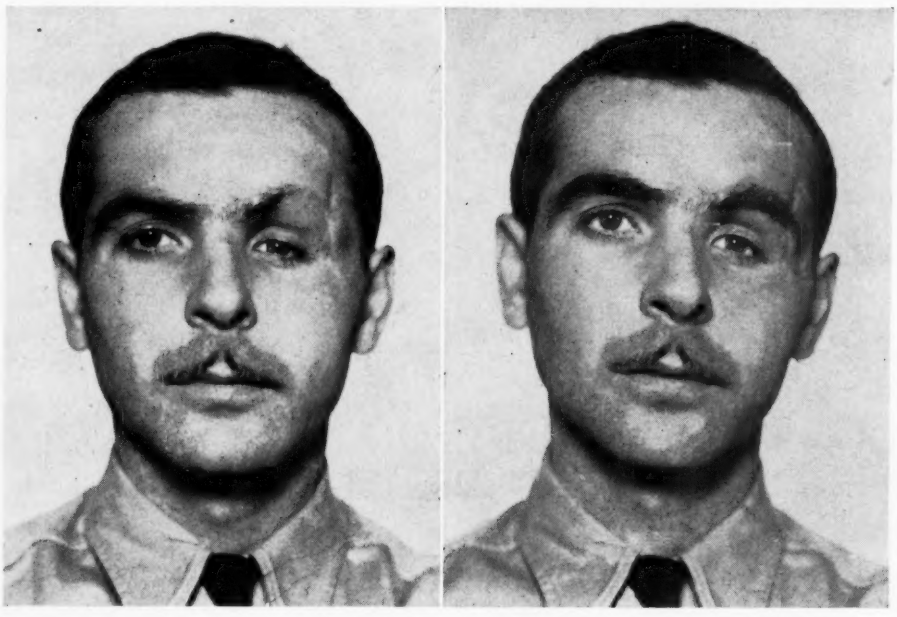

Fig. 12.—Case 1. Left: The restoration of the contour by the use of a chip-bone graft. Right: A cosmetic pencil was used to reconstruct the eyebrow. The ptosis of the upper left eyelid resulted from a partial destruction of the levator and superior oblique muscles.

It is important to note that the fragments must be sufficiently pressed together so that the cast chip-bone graft will emerge from the mold as one solid mass. Failure to do this results not only in irregularities on the surface of the graft but also in a flattening of the curvature of the graft, which would prove detrimental to the contour restoration.

Immediate follow-up studies on the three cases reveal the following facts:

1. Cases 1 and 2 presented a resistant, firm, and shapely contour restoration after five and four months respectively. In Case 2 a few small irregularities that were present have become smooth. Roentgenographs taken after the operation suggest that the numerous fragments have become knitted into a uniform mass.

2. Case 3 represented a large defect which was filled by the cast bone graft. However, because of the fact that the mass was not sufficiently compressed, some flattening took place. This was easily corrected in a second surgical operation. In such a large defect it should also be possible to incorporate in the bone mass one or two tantalum-strip supports or a tantalum wire mesh if necessary.

Fig. 13.—*A* and *B*, Case 3, Lt. B, before the operation.
C and *D*, The same patient 27 days after the operation.

CONCLUSIONS

The method described was used in three cases. The first operation of this type was performed in August, 1943. It is still too early (December, 1943) to predict whether the grafts now in place will give ultimate clinical satisfaction. The final clinical results obtained in these cases will be reported later.

The technique represents a considerable amount of work in preparing the casts and molds before the operative procedure, but the surgery is thus simplified. The shaping of the graft is done by the mold, and the contour, although it must be predetermined, may be excellent.

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Erratum

In the article "A Solid Medium for the Transportation of Delayed Gonococcus Cultures" by Nell Hirschberg in the March issue of this JOURNAL **29**: 314, 1944, page 315, line 37:

0.24 per cent Nile blue A should read 0.024 per cent Nile blue A.

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BOOK NOTICES

The Compleat Pediatrician*

THIS compendium of pediatric facts and figures is an outgrowth, we are told in the preface, of a notebook of easily forgotten data concerning the diagnosis and treatment of the afflictions of children, which was begun a quarter of a century ago. In the interim it has grown to hold a vast amount of information on all phases of child care and development, for both the sick and the well child. It is avowedly a purely *practical* guide, containing little or no theory, and occasionally statements are made that for lack of discussion seem to need further qualification than that with which they are presented.

The sections on laboratory methods, nursing care, and growth and development are exceedingly useful. The book's greatest value in the diagnosis of children's diseases is that it tells the student or physician what to look up somewhere else in a given case. Treatment is very well handled, with the above reservations.

VICTOR C. VAUGHAN III

*The Compleat Pediatrician. Practical, Diagnostic, Therapeutic, and Preventive Pediatrics. For the use of Medical Students, Interns, General Practitioners, and Pediatricians. By Wilburt C. Davison, M.A., D.Sc., M.D., Professor of Pediatrics, Duke University School of Medicine, and Pediatrician, Duke Hospital. Formerly Acting Head of Department of Pediatrics, the Johns Hopkins Hospital, and Member American Board of Pediatrics. Fellow American Academy of Pediatrics and American College of Physicians, Member American Pediatric Society, and Division of Medical Sciences, National Research Council. Fourth Edition. 256 pages, $3.75. Durham, N. C., printed by Seeman Printery for Duke University Press, 1943.